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GENOTYPE AND COMPETITIVE ABILITY OF TRIBOLIUM SPECIES

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The classical investigations of Park and his associates on interspecific competition in laboratory populations of Tribolium have been reviewed on a number of occasions (Park, 1954; Neyman, Park and Scott, 1956). The fundamental findings reported include the observations that under any environmental regimen (specified by temperature and humidity) investigated, one or the other of two competing species (*T. confusum* and *T. castaneum*) is completely eliminated. In certain environments the outcome is determinate. Thus, when temperature and humidity are both relatively high, *T. castaneum* invariably is the surviving species; when temperature and humidity are low, *T. confusum* persists whereas *T. castaneum* disappears (though it may be noted that under this particular regimen this species does not survive even in absence of competition). Under intermediate conditions, the outcome for single cultures is indeterminate. That is to say, within a given environment, *T. confusum* will be the winner in a certain proportion of cultures, but it is impossible to predict *a priori* in which ones it will be successful. Neyman, Park and Scott (1956) have analyzed this situation on basis of a stochastic model. Presumably their approach was based on the assumption of genetic uniformity of the foundation stocks in the competing cultures. More recently, Park, Mertz and Petrusewicz (1961) have addressed themselves more directly to the genetic basis of competitive ability, though so far only their preliminary studies have been reported.

Earlier, Park and Lloyd (1955) examined one aspect of this question. Assuming that genetic differences in competitive ability exist, it is reasonable to suppose that the cultures in which a given species is the winner have a better than average genotype for this trait. In such a case the elimination of the losing species would be accomplished more rapidly or more frequently, if the winners are once more put in competition with average representatives of the other species. This hypothesis was tested by Park and Lloyd with negative results: the occasionally winning *T. confusum* populations (at 29°C and 70 per cent relative humidity) were no more successful on a rerun of the competition experiment thay they were in the original test. In other words, selection between cultures of *T. confusum* did not appear to have any appreciable effect on the outcome of competition between the two species.

Since it seems improbable that genetic variation in competitive ability does not exist, we have attempted to reinvestigate the question, both by undertaking a selection program analogous to that of Park and Lloyd (1955) and by studying the competitive behavior of inbred lines of the two species. These experiments are the subject of the present report.

MATERIAL AND METHOD

Synthetic populations of the two species (*T. confusum* is hereafter designated by *CF*, and *T. castaneum* by *CS*) were established by a systematic program of reciprocal crosses between several strains of different origin, which were eventually combined into single interbreeding groups for each of the species. The initial crosses (20 mass matings for *CF* and 17 mass matings for *CS*) were made by mating the various strains in different combinations. The F individuals obtained from the interstrain crosses were then

TABLE 1

Number of individuals used in original crosses for establishing the synthetic populations

Species	Source of strains	Males	Females
T. confusum (*CF*)	California	99	114
	Illinois	53	99
	Indiana	99	158
	Kansas	103	138
	Minnesota	19	14
	Washington	92	141
T. castaneum (*CS*)	California	31	30
	California	25	44
	Georgia	25	39
	Illinois	10	9
	Indiana*	51	56
	Indiana	25	54
	Kansas	15	60

*A laboratory strain carrying the *sooty* gene (see text).

mated together in five and four mass cultures respectively. For each of the two species the F_2's produced were combined into a single population, their offspring being the foundation generation of the synthetic culture. The numbers of individuals contributed by each strain to the initial crosses are shown in table 1. They originated from laboratory stock sent to us from the locations indicated, except for the California strains which were established by us from specimens taken in poultry feed bins and local mills.

In order to facilitate discrimination between adults of the two species, an autosomal recessive gene for black body color (sooty) was incorporated into the *CS* synthetic population. This was done by using in all initial crosses an Indiana laboratory strain (kindly supplied to us by Dr. A. E. Bell) carrying this gene, and selecting only black beetles of the F_2 to become parents of the foundation generation of the synthetic population.

The synthetic populations were established in September, 1958. Since then they have been propagated in mass cultures maintained in half-pint bottles with no shortage of food. For each species, every three months approximately 50 adults out of each of three such cultures are mixed together and redistributed among three bottles with fresh medium. The pre-adult stages from the old bottles are discarded. Sexed pupae from the mass cultures were used to initiate an experiment involving synthetic populations.

As soon as the synthetic populations were established, a number of inbred lines of both the *CF* and *CS* species were initiated from a number of single pair matings. These lines have been maintained since by duplicate brother × sister matings in every generation. If the first of these matings is successful in producing offspring, the second one is discarded. Otherwise the second is used to propagate the line. In other words, slight selection for reproductive ability is introduced in propagating the inbred lines. The first of the competition experiments described here was started from the 13th generation of brother × sister matings. At that time 22 out of the initial 24 *CF* lines and exactly the same number and proportion of the initial *CS* lines were extant.

All of the maintenance and competition cultures described here were kept under Park's (1954) Treatment III, that is, at a temperature of 29°C and relative humidity of 70 per cent. A commercial Jamesway poultry incubator converted to these conditions was used. The competition cultures were kept in 9.5 × 2.3 cm. vials containing eight gms. of stone-ground sifted (65 mesh) whole wheat flour plus five per cent of dried yeast. They were transferred to fresh vials approximately every 30 days. Two basic differences (in addition to the important modification described later) between Park's and our methods should be noted: (1) instead of starting the populations with two pairs of each species, ten pairs of *CS* and *CF* were used in mixed cultures, while the single-species experimental cultures were started from 20 such pairs; (2) during the monthly census at the time of transfer only adults were counted.

PRELIMINARY EXPERIMENTS

The first experiment undertaken was (with the exceptions in technique noted) identical in design to those carried out by Park (1948 and 1954), Kennington (1953), and Park and Lloyd (1955). In these four competition experiments *CS* was found to be the winner respectively in 12 out of 18, 24 out of 28, 19 out of 20, and 9 out of 10 replications. In our first test, on the other hand, *CS* may be considered to have been successful in all replicates. It is highly likely that, when the competition cultures are started with ten rather than with only two pairs of beetles of each species, the probability of having a culture contain a relatively inferior *CS* genetic composition and a relatively superior *CF* one is greatly reduced. Hence the failure of *CF* to be a winner in any of the replicates is not particularly surprising.

In figure 1 the heavy dashed line shows the mean unweighted percentage of the total adult population represented by the *CF* component of it. The

two light dashed lines indicate the *CF* proportions in single cultures in which this species was respectively the most and the least successful. It should be noted that some cultures had to be discarded because of sporozoan infections (one each at the 19th, 20th, 21st, and 22nd census). The two species of parasites (kindly identified for us by Dr. J. Weiser of Poland through the courtesy of Dr. Y. Tanada of the University of California Department of Insect Pathology) were, more commonly, *Nosema whitei* (see West, 1960) and, more rarely, *Farmocystis tribolli.* Future references to infection refer largely to the former species.

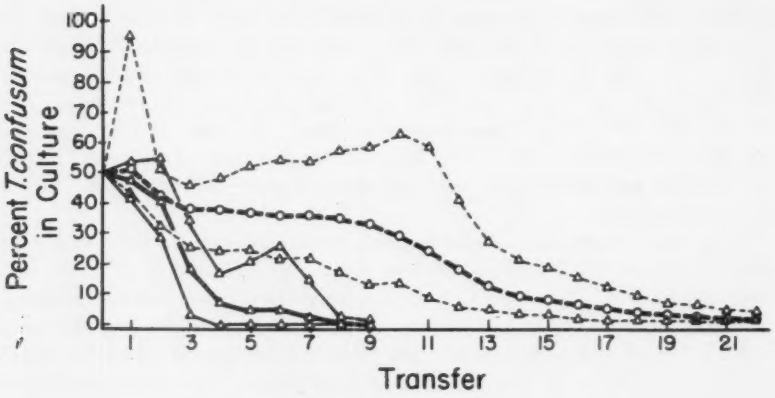

FIGURE 1. The proportion of *CF* adults at each census. The dashed lines represent the first experiment in which all life stages are transferred to fresh vials every month. The solid lines represent the second experiment in which the adults are discarded at every transfer. The heavy lines are unweighted averages of 20 cultures (except for the last four transfers in the first experiment in which 19, 18, 17, and 16 cultures remained respectively). The light lines show the individual cultures in which *CF* was respectively the most and the least successful.

When the experiment was considered terminated, only four of the 16 remaining cultures had any *CF* beetles left, in the respective proportions of 1/198, 3/134, 1/124, and 2/94 adults. The average length of time for total elimination of *CF* is very close to that reported by Park. Since, however, it was clear from his results that each selection cycle contemplated would require more than 20 months, in an attempt to find means to shorten this interval, another experiment was started simultaneously with the one just described.

In the standard technique developed by Park, all life stages (eggs, larvae, pupae, adults) are transferred to the fresh medium. The modification we tried was to discard adults after each monthly census and to transfer only the preadult stages to the fresh vial. The results of 20 replicates for this test are also shown in figure 1, with the heavy solid line representing the average of 20 cultures and the light lines the *CF* proportion in vials

containing the most and the least successful competitive population of this species.

It may be readily seen that whereas the final outcome was the same in this experiment as in the previous one, the length of time it took for complete elimination of *CF* was greatly decreased. At the eighth census only two cultures contained single *CF* adults; at the ninth census none did. All 20 cultures survived the duration of the experiment.

The shortening of the interval before the decisive outcome is reached in this kind of experiment must very likely depend on the fact that the endpoint need not wait on the death of the last adult survivor of the losing species. It is also probable that one of the effects of this modification is to reduce somewhat cannibalism of eggs and pupae by adults. The modified procedure was deemed to be suitable for the purposes of this study and was used henceforth.

There are some points in connection with this technique that may be noted. In the first place, under the standard Park method and this regimen, the number of adults in the *CS* single-species populations is generally found to be higher than in the *CF* single-species populations (see Appendix table 3 in Park, 1954, with which our limited observations show close agreement). Under the modified technique, no clear-cut equilibrium values for each census appear to be established. Rather, in many cultures fluctuating high and low successive monthly counts are observed (this may be seen from figures presented later). Furthermore, *CF* strains appear to have higher production of adults in single-species cultures than do *CS* populations, although in mixed cultures they are losers. This observation is documented by tables appearing in a later section. The reason for this behavior is not immediately clear. It should also be noted that with the modified technique the culture with the highest proportion of *CF* beetles at one census is not necessarily the same as the one at the next census. Furthermore, occasionally a vial containing no adults of a given species is found to have some at the next census, since eggs, larvae or pupae of this species may still exist in the culture. The convention therefore has been adopted for future studies that three successive zero counts of beetles of a species were to be recorded, before it is considered to have been eliminated from the particular culture (though the time of the elimination, of course, is deemed to be that of the first of such series of zeroes). Figure 2 shows the proportions of the cultures which contained *CF* adults at each census in the experiment just described.

At the seventh census, the four most successful *CF* populations contained 12, 8, 6 and 3 adult beetles respectively. Instead of being discarded they were used to start a new *CF* strain. Presumably, should a genetic basis for competitive ability exist, the strain thus reconstituted should be superior to the original *CF* synthetic population.

This possibility was tested by a competition experiment between the new "rescued" *CF* strain and representatives of the *CS* synthetic population. Figure 3 presents in the form of the dashed line the average results ob-

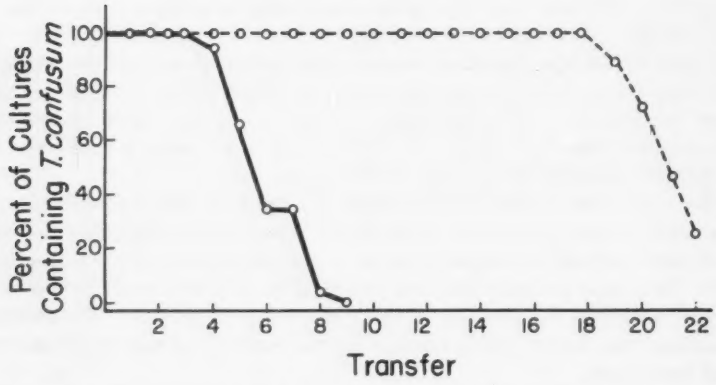

FIGURE 2. The results of the experiments portrayed in figure 1, represented as percentage of the 20 (or less where it applies) cultures containing *CF* adults at each transfer. The dashed line refers again to the first experiment and the solid line to the second.

tained on 20 cultures (reduced by infection to 19 at the sixth transfer). Comparison with the solid line (the second experiment described earlier, that is, identical with the heavy solid line in figure 1) suggests that selection might have been of some effect. Furthermore, one of the 19 surviving cultures at the time of the last census had had three successive counts of zero for *CS*, that is, a winning *CF* appeared.

This somewhat promising first step in selection for a successful *CF* genotype was, however, brought to nought. A second "rescued" *CF* strain was established from the 93 *CF* adults of the *CF* winner crossed with 23 + 12 + 17 other *CF* beetles from the three next most successful populations of this species, all coming from the sixth transfer. This strain was once more put in competition with the synthetic *CS*. As shown by the dotted line in figure 3, the latter made short shrift of the presumably improved *CF* strain. At the third transfer, only six of the cultures had any *CF* beetles in the respective proportions of 1/15, 3/85, 2/43, 4/126, 1/24, and 1/189. At that time signs of infection appeared in some of the vials. It was further noted that the infection focus was the *CF* stock which was the winner in the previous test. Indeed, since we found that in single-species cultures *CS* is much more susceptible to *Nosema* than *CF*, the presumptive *CF* winner may have achieved its success through elimination of *CS* by the infection. Rather than risk further spread of the parasites, all of the cultures in this experiment were discarded.

Although the presence of infection casts some doubt on the possibility of drawing rigorous conclusions from this series of experiments, the results do not seem much more encouraging than those obtained by Park and Lloyd (1955). Presumably the type of selection attempted would be successful largely in the presence of additively genetic variability for competitive

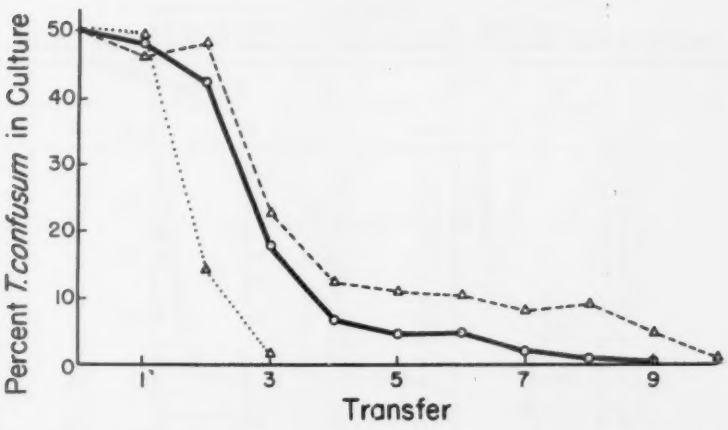

FIGURE 3. Average survival of *CF* in competition with the *CS* synthetic population. The solid line is identical with the heavy solid line of figure 1. The dashed line represents the results of the first cycle of selection of *CF*, and the dotted line those of the second. See text for further explanation.

ability. Dominance (particularly overdominance) and epistatic deviations may make the demonstration of genotypic differences in this property by this method somewhat more uncertain. An investigation of differences in competitive ability between inbred lines would appear to be a more promising approach and was hence undertaken.

EXPERIMENTS WITH INBRED LINES AND CROSSES BETWEEN THEM

A number of the inbred lines mentioned in the section on material and method have been surveyed with respect to their fecundity by Dr. John W. Crenshaw, whose manuscript on this study is in preparation. One more (designated as *CF* 11 and *CS* 2) and one less (designated as *CF* 9 and *CS* 12) productive line from each species were chosen for this experiment. F_1 crosses between these lines were also made, so that for each species three populations were available: *CF* 9, *CF* 11, *CF* 9 × *CF* 11 (designated as *CF* X), and *CS* 2, *CS* 12, and *CS* 2 × *CS* 12 (designated as *CS* X). Nine sets of ten competing cultures each were initiated in a three by three design with each of the *CF* lines competing with each of the *CS* ones. In addition, ten single-species cultures of each of the six populations were carried as controls on the production of adults. The mixed cultures were started with ten adult pairs of each species, the single-species cultures with 20 pairs. Whenever infection was detected, a culture was discarded (none in the first four transfers). When elimination of one species occurred in any whole set, it was discarded. The numbers of cultures on which the data to be presented are based are shown in table 2. With the exception of set G, which on the ninth transfer was found to be infected in most cultures, the element of parasitism did not interfere with the results to be presented.

TABLE 2

Number of cultures on which data in tables 4–5 and figures 4–6 are based

Set no.	Line	Transfer					
		1–4	5	6	7	8	9–12
...	*CF* 9	10	10	10	10	9	...
...	*CF* 11	10	10	10	10	10	...
...	*CF* X	10	10	10	10	10	...
...	*CS* 2	10	8	8	8	8	...
...	*CS* 12	10	10	10	10	8	...
...	*CS* X	10	10	10	9	9	...
A	*CF* 9-*CS* 2	10	9	9	...	...	...
B	*CF* 9-*CS* 12	10	10	10	8	...	...
C	*CF* 9-*CS* X	10	10	...	...	...	...
D	*CF* 11-*CS* 2	10	10	10	10	...	...
E	*CF* 11-*CS* 12	10	10	10	10	10	10
F	*CF* 11-*CS* X	10	10	5	5	5	...
G	*CF* X-*CS* 2	10	10	10	9	9	...
H	*CF* X-*CS* 12	10	10	10	10	10	9
I	*CF* X-*CS* X	10	7	7	7	4	...

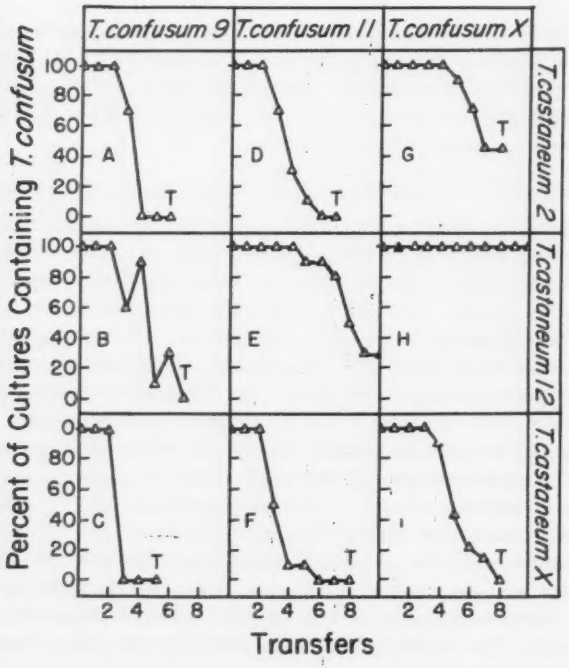

FIGURE 4. Percentage of cultures (initially ten per set) in which *CF* survived. T indicates the terminal census for the set. See table 2 for number of cultures per set.

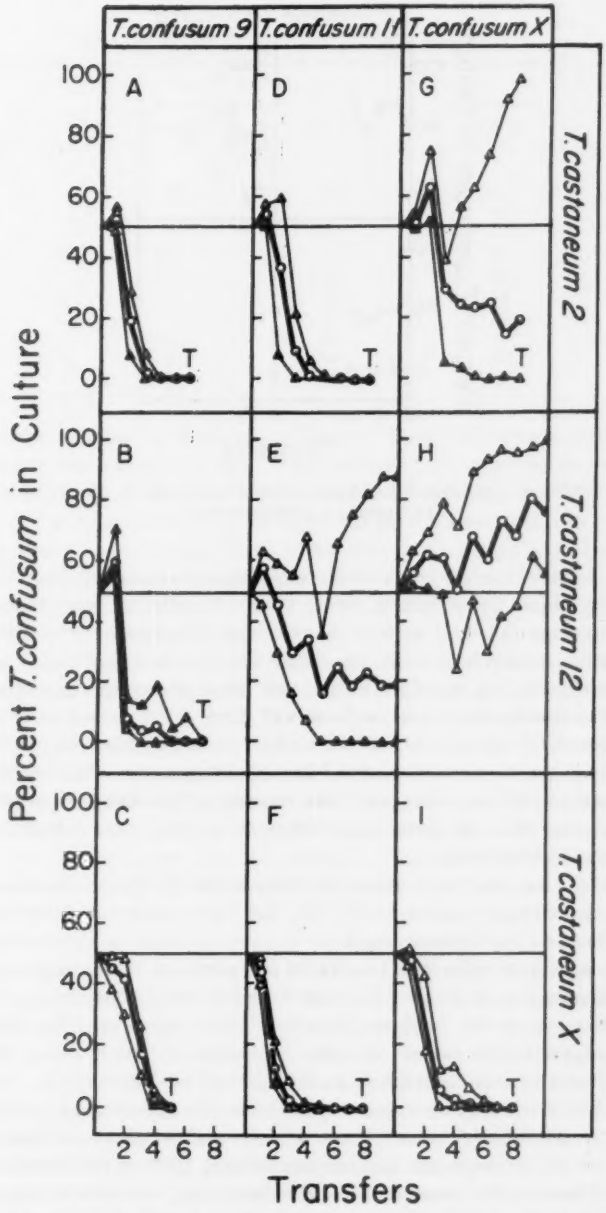

FIGURE 5. Survival of *CF* in competition sets A–I (see table 2). The heavy line in each instance represents the unweighted average per cent *CF* beetles in cultures. The light lines represent the individual cultures in which *CF* was respectively the most and the least successful. T indicates the terminal census for the set.

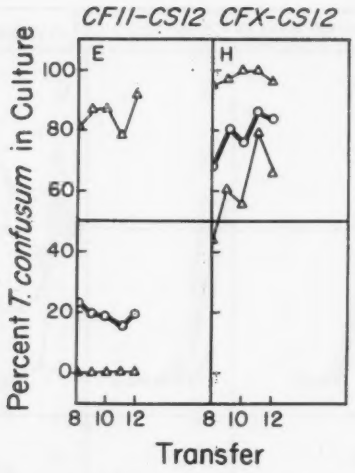

FIGURE 6. Continuation of figure 5 for sets E and H. See caption to figure 5 for explanation.

Figure 4 shows the progress of the experiment in terms of percentages of cultures in which *CF* survived, while figure 5 presents the information in terms of average (as well as highest and lowest) proportions of *CF* beetles in each set. It may be readily seen that *CS* of hybrid origin was very successful in eliminating rapidly all *CF* lines. When the competition was among inbreds, the results were not as decisive. Line *CS* 2 proved to be superior to both inbred *CF* lines, but was not as successful in competing with *CF* X. At the time set G was discarded, five of the cultures had no adult *CF* beetles in them; the percentages in the remaining four were respectively 8.5, 18.6, 47.6, and 98.4. It seems not unlikely that in the last culture *CF* might have emerged victorious.

Line *CS* 12 was the least successful one of the *CS* lines. It was superior to *CF* 9, to a lesser extent to *CF* 11, but was clearly unsuccessful competing with the *CF* of hybrid origin.

By the eighth transfer the results of all sets but three were definitive; set G, as noted, was discarded; sets E and H were continued. The later fate of these is shown in figure 6, which gives the results for these sets from the eighth to the twelfth transfer, and table 3, which shows the status of the cultures at that point (they are being continued currently).

Three kinds of results may be observed: (1) determinate, with *CS* the winner, (2) determinate, with *CF* the winner, and (3) indeterminate, with *CS* superior on the average but with an occasional *CF* win (or probable win in set G). There would seem to be little doubt that the differences between sets are, indeed, genetic in origin, a conclusion supported by the final experiment in the present series reported later.

TABLE 3

Census of adults in sets E and H at 12th transfer

Set	E		H	
Line	*CF* 11	*CS* 12	*CF* X	*CS* 12
Culture no.				
1	0	68	31	16
2	...	...	72	19
3	1	40	77	30
4	81	7	90	4
5	0	19	86	5
6	0	25	114	18
7	0	44	113	33
8	138	33	85	8
9	0	38	77	17
10	0	35	146	6

One of the interesting features of figure 5 is the difference in the variation between replicates of a set. The uniformity of behavior in sets C, F, and I, where *CS* X is an easy winner, is rather striking. Sets A, D, and B in which the same outcome may be observed also shows this. Sets G and E naturally show greater variability, since here both losing and winning (or nearly so) *CF* populations are present. But set H, where *CF* X seems to be the winner throughout, shows a wide range of difference in the proportions of *CF* beetles for most of the experiment.

Tables 4 and 5 give cumulative counts of adults in the various sets. The first transfer represents largely the adults with which the cultures were started; hence it is not included. The data are given in a cumulative fashion starting with the second census. This method of representation appears to be more informative than giving the results at each transfer, because (as may be seen in figure 5) of the up and down alternations in successive counts, occurring as a result of the removal of adults at every census.

It may be readily seen that the ranking of competitive ability of lines within both species is identical with the ranking of productivity of adults (*CF* X, *CF* 11, *CF* 9, and *CS* X, *CS* 2, *CS* 12 respectively). This is contrary to the widespread inverse correlation between productivity and competitive ability, designated by Gustafsson (1951) as the *Montgomery effect*, which, however, appears to exist when the behavior of the two species, rather than lines within each, is compared. Under our technique, *CF* is enormously more productive than *CS* in single-species cultures, in spite of its relative lack of success as a competitor (as noted earlier, this is not true in the data of Park, 1954, though it appears to be so for another regimen studied by Neyman, Park and Scott, 1956).

It may also be seen that, generally speaking, whenever *CS* is the winner, the number of *CS* adults produced in mixed cultures is the same as in the single-species vials. On the other hand, even the most successful *CF* (for example, *CF* X competing with *CS* 12) in competition does not reach the level of a third of the number of adults that the same line produces when

TABLE 4

Average cumulative production of *CF* adults in single species and mixed cultures (8 gms. of flour)

Line	Number summed over transfers	In single species cultures	In competition with		
			CS 2	*CS* 12	*CS* X
CF 9	2–5	444	16	14	4
	2–6	577	16	15	...
	2–7	657	...	15	...
	2–8	752	...	...	...
CF 11	2–5	950	42	94	22
	2–6	1136	42	112	22
	2–7	1285	42	123	22
	2–8	1504	...	148	22
CF X	2–5	1101	169	309	46
	2–6	1305	183	366	46
	2–7	1449	190	440	47
	2–8	1661	201	507	47

TABLE 5

Average cumulative production of *CS* adults in single species and mixed cultures (8 gms. of flour)

Line	Number summed over transfers	In single species cultures	In competition with		
			CF 9	*CF* 11	*CF* X
CS 2	2–5	266	235	237	199
	2–6	337	299	274	244
	2–7	379	...	333	280
	2–8	443	...	...	323
CS 12	2–5	244	240	188	187
	2–6	287	286	233	217
	2–7	333	328	265	241
	2–8	378	...	303	267
CS X	2–5	405	389	400	355
	2–6	465	...	495	435
	2–7	516	...	551	514
	2–8	572	...	620	569

alone. This question, as well as a variety of others regarding the physiological basis of competitive ability are, of course, still open, and will, no doubt, receive illumination from the series of studies initiated by Park, Mertz and Petrusewicz (1961) on different strains of Tribolium, and, we trust, from investigations on selected populations and inbred lines under way in our laboratory.

In the meanwhile, the significant fact about the data reported lies in the demonstration of the dependence of competitive ability on genotype. This is not a surprising conclusion. Yet it does not seem to have been previously

demonstrated. Indeed, as noted, the results of Park and Lloyd (1955), as well as our own failure to improve the competitive ability of *CF* by selection seemed to give contrary evidence. It is not unlikely that the explanation of this discrepancy lies in the possibility that competitive ability is *genotypically* rather than *genetically* determined (see Lerner's, 1958, account of this terminology). In other words, the action of the *CF* alleles responsible for success in competition with *CS* under the regimen of these experiments, is not independent of the rest of the genotype in which they are found. Both dominance (and particularly overdominance) and epistatic effects may play a significant role in competitive ability. Comparative failure to observe improvement from selection, while inbred lines and hybrids are readily demonstrated to differ in competitive ability, may find an explanation in such a mechanism. At this stage, however, this conclusion must be regarded as speculative.

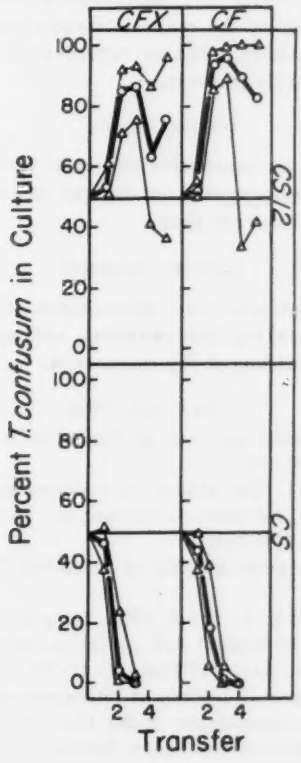

FIGURE 7. Survival of *CF* and *CF* X in competition with *CS* and *CS* 12. See text for further explanation.

The final experiment in this series was carried out in order to verify the results reported. Four sets of ten competing cultures each were initiated. These consisted firstly of *CF* X from the seventh transfer of set H which were placed in competition with the *CS* 12 line, by now in its 18th generation of brother × sister mating. Secondly, the same *CF* X extraction was placed in competition with the synthetic *T. castaneum* stock (designated as *CS*). In the third set the competitors were the synthetic *T. confusum* stock (designated as *CF*) and *CS* 12. Lastly, the fourth set was a control replication of the competition between *CF* and *CS*.

Figure 7 shows, in the same manner as figures 5 and 6, the direction in which this experiment is going, its results being in accord with expectation. Namely, *CS* tended to eliminate *CF*; *CF* X was successful in competing with *CS* 12 (approximately to the same degree as in the earlier experiments), but was unable to hold its own against *CS*. Perhaps, the only new information obtained here is that the inbred *CS* 12 is unable to withstand the competition of *CF*. Apparently, the synthetic *T. confusum* population is at least as good a competitor as the one which originated from a cross of two inbred lines, but which has undoubtedly by the seventh transfer lost some of the heterosis it was endowed with initially. Further experimental analysis of this situation may be of considerable interest.

CONCLUSIONS

Genotypic differences in competitive ability of *Tribolium confusum* and *T. castaneum* have been demonstrated by the use of inbred lines and strains originating from crosses between them.

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INBREEDING, HETEROSIS AND INFORMATION THEORY*

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I. INTRODUCTION

The empirical knowledge that inbreeding of plants and animals is likely to reduce the vigor of the offspring and that outbreeding often has the opposite effect long antedated the birth of the science of genetics. This reduction takes many forms. It may be in size, longevity, activity, fertility, uniformity or in various degrees and combinations of all these and many other characters. The effects of inbreeding have been extensively studied experimentally during the past half century from the point of view of both the practical breeder and the theoretical geneticist (Gowen, 1952). There are similarities in the phenomena whether one observes them in maize, in chickens or in Drosophila, but there is still no general theory which accounts for them all in a really satisfactory way.

A similarly ancient problem is that of the nature of life itself and here also we still lack an epistomological consensus. Can life be explained by means of the same principles which apply to the inanimate world? The vitalist-mechanist front of the nineteenth century War between Science and Religion is no longer very active, but the philosophical conflict is by no means resolved. Lively skirmishes are being fought today between the biochemist and the biologist (Commoner, 1961). An attempt which has arisen in the past two decades under the influence of information theory and cybernetics to analyze the organism as a complex automaton shows promise of synthesizing these opposing positions (Elsasser, 1958). Some recent experiments with inbred and randomly breeding populations of *D. melanogaster* suggest that the application of concepts from this latter method of analysis may lead us to a general theory of inbreeding effect and heterosis.

II. SOME DATA ON INBREEDING AND HATCHING RATIO IN *D. MELANOGASTER*

D. melanogaster responds to inbreeding with reduced biological efficiency in many ways among which is a reduction in the number of eggs which hatch. Two populations (Sy and OR) kept in cages in the laboratory for the past eight years where the population size has averaged several thousand and breeding has been at random produce eggs with a probability of hatching of about 0.95. Three inbred lines, one (SySP) stemming from Sy and two (I_1 and I_2) from OR, carried for more than one hundred generations by brother-sister mating, give probabilities of hatching of approximately 0.3, 0.5 and

*This paper is dedicated to Professor L. C. Dunn in recognition of his long and distinguished career and in gratitude for the understanding and encouragement which once helped a political scientist climb the fence into biology.

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TABLE 1

Hatching ratios of five populations of *D. melanogaster*

Population	Sy	OR	SySP	I_1	I_2
Proportion hatching	.9398	.9657	.3151	.5153	.8016
Total number	5050	4593	4490	1638	1754

0.8 respectively. (See King, 1961, for details of technique used.) Table 1 gives precise data for this character.

The randomly breeding and the inbred populations differ strikingly also in the variability of these proportions. The measurements were made by taking samples of 50 eggs and scoring the number hatched after 48 hours. The accumulated scores of many such samples provide two measures of variability. There is first the theoretical sampling variance of the ratio of eggs hatched to total, equal to pq/n. Another variance can be computed from the individual scores of a number of samples of 50—say those taken within a given month. If the hatching probability of a population is constant, the variance between samples should consist merely of sampling variance and should approximate pq/50. For the two randomly breeding populations this is substantially the case. If, on the other hand, the probability of hatching changes from day to day, the variance between samples will be higher than the sampling variance based on the ratio for all samples lumped together. This is actually the situation with the inbreds.

To make our comparisons valid and accurate, some statistical explanation is necessary. We are interested in comparing two variances, one theoretical and one observed. Both the F-test and the standard deviation of a variance calculated from χ^2/d.f. assume normal distribution of the observations giving rise to the statistics, an assumption scarcely justified here. It is safer to calculate from the observations themselves a 95 per cent confidence interval for the variance between samples. This can be done by treating every $(x - \bar{x})^2$ within a group of samples as a datum, obtaining a mean and a standard deviation of the mean for these data and calculating 95 per cent confidence limits for the mean. These figures multiplied by $n/n - 1$ will give us the variance between samples and its 95 per cent confidence limits (Levene, 1960). Armed with these statistics, we can ask with respect to any group of samples: Do the 95 per cent confidence limits of the variance between samples embrace pq/50 for the consolidated totals, as they should 19 times out of 20 if the probability of hatching was constant during the period of sampling?

Table 2 gives the pertinent figures for such comparisons and figures 1 and 2 show the relationships graphically. It is strikingly clear that we have one pattern characteristic of the randomly breeding populations and quite another in the case of the inbreds. For the former, in six out of six series of measurements on both populations the 95 per cent confidence interval of the variance between samples includes the value of pq/50, the theoretical

variance of the ratio. For the inbreds on the other hand, there is a strong tendency not only for the variance between samples to be very much larger than pq/50 but also for the 95 per cent confidence interval of the former not to include the latter. In the case of SySP, four of the six confidence intervals fail to include the value of pq/50; in the other two cases the lower confidence limits are only slightly smaller than pq/50. For SPC—a population taken from SySP and allowed to expand in a cage to a size of several thousand—two series of measurements give confidence intervals neither of which extends low enough to include pq/50. For I_1 and I_2, both of two series for each give one confidence interval including pq/50 and one not reaching it. There can be no question that the probability of obtaining confidence limits for the variance between samples which do not include the value for the corresponding pq/n is much greater for the inbreds than for the large, randomly breeding populations. It is obvious also that the variance between samples and its confidence interval for the randomly breeding populations are consistently smaller than the corresponding statistics for the inbreds. The scale in figure 1 is one-fifth of that necessary to plot the data for the inbreds in figure 2.

TABLE 2

Hatching ratio, theoretical variance and observed variance with 95 per cent confidence limits

Population and period	Hatching ratio	pq/50	$s^2 - t_{s_{s^2}}$	s^2	$s^2 + t_{s_{s^2}}$	Number of samples
Sy II '60	.9538	.000881	.000453	.001022	.001591	16
III	.9400	.001128	.000365	.001137	.001909	20
IV	.9133	.001583	.000000	.004210	.008569	15
V	.9513	.000927	.000334	.000852	.001370	16
VI	.9442	.001054	.000561	.001450	.002335	19
VII	.9333	.001245	.000521	.001410	.002299	15
OR II '60	.9670	.000638	.000111	.000741	.001317	11
III	.9490	.000968	.000281	.001578	.002875	20
IV	.9338	.001237	.000159	.001425	.002691	16
V	.9263	.001365	.000000	.002706	.009759	16
VI	.9505	.000941	.000821	.001083	.001345	19
VII	.9667	.000644	.000238	.000495	.000752	15
SySP II '60	.3588	.004601	.003200	.025362	.047520	16
III	.3378	.004473	.010109	.019860	.029611	15
IV	.2970	.004176	.005029	.016062	.027095	14
V	.2738	.003977	.005838	.019433	.033012	13
VI	.3278	.004407	.010046	.022783	.035520	18
VII	.2814	.004044	.003190	.026798	.050406	14
SPC II '60	.4176	.004864	.006712	.020334	.033956	16
III	.3667	.004645	.009839	.036262	.062685	15
I_1 VIII '59	.6580	.004501	.000951	.011217	.021483	11
XI-XII '60	.4455	.004941	.019358	.033225	.047092	22
I_2 VII-VIII '59	.7985	.003218	.006067	.022103	.038139	13
XI-XII '60	.8035	.003158	.002447	.005129	.007811	22

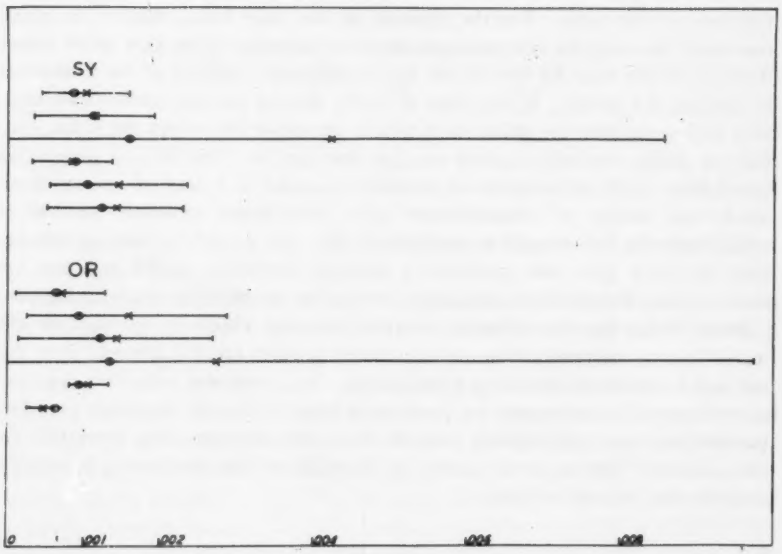

FIGURE 1. Variance of the hatching ratios of Sy and OR for six periods, each based on repeated samples of 50 eggs. Cross marks variance value; line shows extent of 95 per cent confidence interval; circle shows value of pq/50. Numerical data in table 1.

The egg samples on which all these statistics are based were obtained from groups of between 100 and 150 females and about the same number of males kept, for purposes of egg collection, in half-pint culture bottles changed every other day over a period of a week. Coming from groups of females, these samples give no indication of the way in which individual females contributed to the differences between populations in the mean or in the variance between samples of the hatching ratio.

To get information on this point, two types of experiments were carried out. First, single females taken from the egg-laying groups were kept in vials for five days, the daily egg production of each being counted and scored for hatching. Although the collection of eggs from the groups of flies showed no pattern of decline from Monday's collection to Thursday's, single females in vials, even though accompanied by two males, tended to run down in egg production over the same period. By this method one was not getting measurements based on the same processes which were going on in the half-pint culture bottles. Therefore, a second type of experiment was tried. Single females, each accompanied by two males, were extracted daily from the larger group and each was scored for one day's egg production and its hatching ratio.

The results of the second set of measurements are given in table 3. The hatching ratios are consistent with those obtained from egg samples collected from the groups of flies; the variances here, however, are not be-

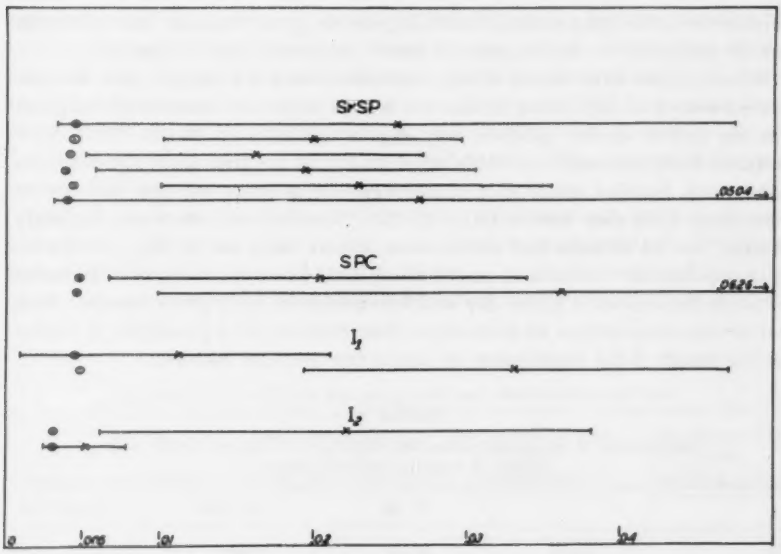

FIGURE 2. Variance of the hatching ratios of four inbred populations for different periods, each based on repeated samples of 50 eggs. Cross marks variance value; line shows extent of 95 per cent confidence interval; circle shows value of pq/50. Numerical data in table 1.

tween samples of equal size to which many females have contributed, but between groups of different sizes laid by individual females. Consequently, they are higher. However, the variance for the randomly breeding population is strikingly lower than that for either of the inbreds. Of the 50 Sy females none were sterile. Among the 50 SySP's 19 produced no eggs that hatched; among the I_2's there were six such individuals. If we eliminate the sterile females and figure the hatching ratios on the remainder, the ratios are still consistent with the overall averages for the populations and, what is more significant, the variances between the hatching ratios of the non-sterile females is still very definitely higher than it is for Sy. For SySP, removing the sterile females actually increases the variance! It is clearly true that

TABLE 3

Mean and variance of hatching ratios of eggs from single females

Population	Total			Number sterile	Sterile subtracted		
	Number of females	$\bar{X}$	s^2		Number fertile	$\bar{X}$	s^2
Sy	50	.9312	.0088	0	...	...	...
SySP	50	.2646	.1149	19	31	.4268	.1165
I_2	50	.6480	.0833	6	44	.7364	.0277

neither the lower proportion of hatching nor its great variance in the inbreds can be attributed to the presence of some completely sterile females.

Moreover, the first series of measurements where the same female was observed over five days showed that one female might lay some fertile eggs on one day and on another produce only eggs which failed to hatch. Of 20 SySP females, three produced no viable eggs on any of the five days. On the other hand, nine females which laid viable eggs on at least one day had one or more days when they were sterile. Of 20 I_2 females, not one was completely sterile, but 14 of them had one or more sterile days out of five. In the inbred populations variance in the hatching ratio is compounded of differences between females on a given day and between days for a given female. Such variability must reflect an instability characteristic of a genotype. It cannot be the result of the segregation of one or two sterility factors.

TABLE 4

Comparison of hatching ratios and variances of Sy and SySP with those of crosses between them

		V '60	VI '60	II-III '61
Sy	ratio	.9513	.9442	
	pq/50	.000927	.001054	
	s^2	.000852	.001450	
	n	16	19	
Sy × SySP	ratio	.8647	.8611	.8600
	pq/50	.002340	.002392	.002408
	s^2	.002575	.003222	.003200
	n	17	19	8
SySP × Sy	ratio	.5588	.4969	.5854
	pq/50	.004931	.005000	.004854
	s^2	.069367	.023900	.032921
	n	7	6	8
SySP	ratio	.2738	.3278	
	pq/50	.003977	.004407	
	s^2	.019433	.022783	
	n	13	18	

The data in table 4 throw further light on this instability of embryonic development in SySP. Here we have a comparison of hatching ratios of Sy and SySP eggs fertilized by sperm of males from their own populations with Sy and SySP eggs fertilized by sperm from the other. SySP sperm in Sy eggs reduce the hatching ratio by something like ten per cent, but the variance between samples does not deviate greatly from pq/50. Sy sperm in SySP eggs raise the hatching ratio by from 50 per cent to 100 per cent but they do not reduce the variance between samples. Even when fertilized by Sy sperm, which are about 95 per cent effective in Sy eggs, the SySP eggs have only about 50 per cent chance of hatching. SySP sperm which are about 90 per cent effective in Sy eggs are less than 90 per cent of 50 per cent effective in their own eggs. Oögenesis in SySP has a low efficiency and produces

numerous eggs incapable of successful development. But the process is very erratic and the percentage of defective eggs varies sharply from day to day. In any given sample of SySP eggs, however, the probability of successful development is greater in an embryo having an SySP-Sy genome than in one fertilized by an SySP sperm. We are dealing with a complex set of interactions and it seems highly likely that these shifting probabilities are susceptible to environmental influences.

One of the most effective environmental influences on development is temperature. Slight variations affect mean hatching time strongly. Do these variations affect the probability of hatching? It has not been possible to control or to measure the laboratory temperature with sufficient precision to

TABLE 5

Correlations involving hatching ratio and mean hatching time

Period	Strains	Mean hatching time with mean hatching time		Mean hatching time with hatching ratio		Hatching ratio with hatching ratio	
		r	n	r	n	r	n
II–III '60	Sy			−0.211	17		
	OR			0.195	17		
	Sy-OR	0.975*	17			0.000	20
II–III '60	SySP			0.244	19		
	SPC			0.209	18		
	SySP-SPC	0.949*	16			0.634*	29
VIII '59							
XII '60	I_1			0.096	18		
VII–VIII '59							
XII '60	I_2			0.091	21		
	I_1-I_2	0.983*	18			−0.008	32
X-XI '60	I_1-SySP					−0.123	14
X-XI '60	I_2-SySP					−0.085	14
IV–V '61	I_{1A}-I_{1B}					0.257	28

*Significant at the five per cent level.

state the exact mathematical relationship between temperature and mean hatching time. Casual observation reveals that when the temperature is low, hatching is delayed. That the different populations respond similarly and precisely to temperature differences is revealed by plotting, for measurements made on the same day, mean hatching time of one population on that of another. The first column of table 5 shows the remarkably high correlation values obtained.

If variations in temperature also produce changes in hatching ratio, we should find mean hatching time correlated with per cent hatching within populations. But as is clear from the figures in the second column of the table, none of the strains tested shows any such sympathetic variation.

Whatever produces variation in hatching ratio, it cannot be primarily minor fluctuations in temperature during embryonic development.

The hatching ratio may, however, be sensitive to the many small environmental differences which even controlled laboratory conditions fail to keep constant. If this is so, we might expect to find correlations in the day to day changes in hatching ratio when comparisons are made between populations. The third column of table 5 gives the results of such calculations. The only combination giving a significant value is SySP-SPC. Actually, this is an intra-population comparison; SPC was taken from SySP after 194 generations of inbreeding only three months before these measurements were made. In the light of this finding, another set of intrapopulation measurements was made. Two separate mass cultures of I_1, labeled A and B, were set up and paired measurements of the hatching ratios were made over a period of seven weeks. In this case, however, there was no significant correlation between the 28 pairs of observations.

III. HATCHING RATIO AND DEVELOPMENTAL EFFICIENCY

Our data tell us that zygotes of a given population have a characteristic probability of completing embryonic development and that this probability has a characteristic stability through time. In large, randomly breeding populations the probability and its stability tend to be high; in inbred populations both are lower. This is consistent with the finding of Dobzhansky and Wallace (1953) that random heterozygotes vary less in viability from culture to culture than artificially produced homozygotes. This they attributed to greater "homeostasis" of the heterozygotes which made them "better able than homozygotes to cope with . . . different environments and to maintain their internal milieu in functional order." But they admitted that "we do not know which physiological process in Drosophila must be maintained constant to enable the development to proceed unimpaired . . ."

Cannon (1932), who developed the concept of homeostasis, thought of it as applying primarily to the processes by which the human body maintains certain constant physical and chemical conditions such as temperature and ionic concentration of the blood. While it is probably true that similar processes act during development, the profound chemical and structural changes which occur in the embryo must require somewhat different performance from the stabilizing forces than do the settled organs of an adult creature, all the more when the changes are taking place in a nutritionally autonomous egg outside the maternal body. When extensive alterations are in progress, it may take quite a different routine to maintain business as usual. Waddington (1957, p. 43) has pointed this out. ". . . it would only lead to confusion," he writes, "to neglect the distinction between the two concepts of canalization of developmental end-state and homeostasis of the level of physiological functioning." The fact remains, however, that there is a difference in the efficiency of the developmental process between the random heterozygote and the artifical homozygote or the inbred.

In the Dobzhansky-Wallace experiments viability was equated with success in reaching adulthood. Larval and pupal, as well as embryonic stages

were involved. This meant that there were very complex environmental influences at work, especially during the larval period—nutritional differences, inter-individual competition, intergenotypic interaction since there were always at least two genotypes in each culture, to mention only a few of the enormous number of factors. In our hatching-ratio data the situation, though still complex, is decidedly simpler. Environmental influences are considerably more limited. Temperature changes and gaseous diffusion are almost the only two processes which can be communicated to the interior of a Drosophila egg. The fact that development can proceed normally in an egg which has been dechorionated in a one-to-one dilution of commercial Chlorox is striking evidence of the insulating property of the vitelline membrane. Since, in these experiments, temperature, humidity and ambient atmosphere were kept very nearly constant, the differences in behavior between eggs must have resulted primarily from reactions between events occurring within them. This is not to say that environment, external or internal, of the parents may not at an earlier time have influenced the nature and composition of the gametes; especially the cytoplasm of the egg. But once fertilization has taken place, the business of development proceeds in what is substantially a closed session. Deviations from good order must be the result of internal dissension.

Certain of the processes of embryonic development are physical, such as displacements resulting from changes in pressure or the orientation of growing cells with relation to tissues already formed, but the fundamental kinetic influences are chemical. The substances forming the egg interact under catalysis of the genome after fertilization to produce the fully developed embryo. Development is a complex series of open chemical systems proceeding toward steady states and interacting in a time sequence to produce the patterned, functioning structure which is the first instar—open systems, even though the egg is largely insulated from the environment, because the cells absorb nutriment from the yolk, gradually consuming it, and rid themselves of waste products which escape through diffusion or accumulate as refuse in the gut. Various models of such chemical systems and their possible interrelations, such as those worked out by Kacsar (1957), can account for many of the phenomena observed by the embryologist and the developmental geneticist—regulation, pleiotropy, buffering, etc. Such models assume that the chemical systems are under genetic control, but chemical kinetics as such cannot account for the pattern, the creode, nor the precise differences between populations in the probability of staying on the course or straying off. As Dobzhansky and Wallace put it, ". . . nor do we know the nature of the gene action that produces the buffered system for the constancy of such process."

IV. INFORMATION THEORY AND AUTOMATA

Contemporary efforts to build a general theory of biological phenomena trace back about thirty years and owe much to the Twentieth Century revolution in physics. In 1933 Niels Bohr suggested that since Newtonian Physics had proved to be a special case of quantum mechanics, perhaps all in-

animate phenomena are special cases within a broader framework which includes living matter and that an understanding of this broader framework might dissolve the animate-inanimate dichotomy. Schrödinger in *What is Life?* (1946) and Teilhard de Chardin (1959) in *The Phenomenon of Man* both said essentially this although in very different ways.

As a part of the breathless pace of scientific development in recent decades, rapid and substantial progress toward this broader theory is now under way. Since 1940, quantum physics, the telephone, radio, television and high speed computers have cooperated to produce a mathematical analysis of information and a theory of automata. The work of Shannon (1949) and Wiener (1948), at first applied largely to telecommunications and servomechanisms, soon became recognized as applicable to the functioning of the nervous system and later to metabolism, development and genetics.

Probably the most comprehensive and penetrating discussion of this whole subject is that of Elsasser (1958, 1961). Biological entities, he points out, are not subject to the predictive procedures of the physicist for (1) they are systematically unhomogeneous and (2) they do not exist in infinite classes. They appear to be, however, amenable to analysis as information-manipulating automata. The old vitalist argument that "purposive" action distinguished living from non-living matter can now be exorcized, for actions indistinguishable from "purposive" are performed by cybernetic devises of only mild complexity. Nevertheless, there are as yet unexplainable phenomena in living things. Chief among these are the incredible efficiency with which they store information for ready reference and the methods by which they prevent the accumulation of error.

The astonishing rate of advance in this whole field is illustrated by the fact that Elsasser's well grounded and sophisticated work of 1958 does not contemplate DNA as the carrier of the genetic code; his 1961 paper, of course, does. On the matter of minimizing the accumulation of error, a report given at Dayton, Ohio, in September, 1960, is summarized (Lipetz, 1961) thus: "A. N. Verbeek showed how to produce reliable computation with a noisy, unstable neuron . . . He used triplet networks. . . . the output of all the triplets goes to a neuron which acts as a majority decider, taking the outputs, comparing them and deciding which is correct according to the output signals of the majority. If each neuron is capable of producing an error five per cent of the time, the upper limit of the probable error of such a net can be made less than one in one million by a combination of just 30 such redundant triplets. This work is of great significance, since up to now the only theoretical method of increasing reliability has been to put many elements in parallel whenever one element has been used in the original net. Von Neumann [whom Elsasser quotes] has shown that to achieve a reliability of one error in a million by this older method with neurons which produce an error five per cent of the time would require a net of about 20,000 neurons." This does not mean that we can now explain just how cells and organisms avoid chaos resulting from accumulating error, but it makes the problem appear much less formidable than it did only two or three years ago.

Several other recent papers have made signal contributions to this field —among them a collection published in *The American Naturalist*, 93 (1959) No. 871, pp. 209–254, especially E. Racker's "Multienzyme Systems" and H. Quastler's "Information Theory of Biological Integration."

To summarize at the level of extreme generalization and simplification: classical physics divides events into the possible and the impossible; quantum physics classifies them as more or less probable; the living system manipulates probabilities and consistently produces the less probable. The living system achieves this by the integration of elements into an entity in which they are related to each other through output-input reactions modified by feedback. This entity, the organism, operates as a self-sustaining and self-reproducing automaton. It contains information which determines its maintenance, behavior, reproduction and development and this information is of exactly the same sort as any other information —from the formula for computing e to an episode of The Untouchables. It can be analyzed mathematically and translated from code to code because its essence is a patterned deviation from randomness or maximum entropy.

The information in an organism—say a robin—is composed of all those units of its chemical and geometric construction plus those dynamic processes going on within it which are necessary to its continued existence as a robin. The dynamic processes are of equal importance with the chemistry and the spacial configuration. They correspond to the information in dynamic storage in a computer which vanishes if the current is turned off. To build a living organism by recreating its physical structure and chemical composition would be equivalent to building a computer and completing it midway in its solution of a given problem. The information in a living robin is responsible for its physical and physiological characteristics and for its behavior. This is what covers its body with feathers in a given color pattern, what causes it to seek and eat worms and berries and to digest and assimilate them in its characteristic way, what causes it to migrate with the change of seasons and to mate, build a nest, lay eggs, incubate them and rear its young.

The information which constitutes a robin could be coded. If we knew how, we could express it in a series of binary digits although it would necessarily be a long one. Thus accurately expressed, it could be used to construct another robin. We do not know how to code this information nor how to use such a coded message to construct a robin. But the robin does. It produces eggs in which the necessary information has been coded in an entirely different form. An egg no more resembles a robin than a set of blue prints and a contractor resemble a house, but in the proper circumstances the one produces the other.

There are two fundamental concepts of information theory which must be described in some detail before its application to biology can be clearly explained. These are redundancy and noise. Any piece of information can be translated into a precise quantity of either/or choices and the logarithm to the base of two of the number of such choices is the number of units or

"bits" (binary digits) by which any piece of information can be measured. Any message has a minimum number of bits necessary for its accurate expression, but as coded, it may contain more than this minimum number of units. Without going into the mathematics involved, redundancy is a measure of the excess of information in a message over the necessary minimum. It is unfortunate that this term was selected for the concept for, to too many American intellectuals who suffered traumatically in freshman composition, redundant is a dirty word. Insurance would have had a pleasanter and ethically less outrageous connotation.

The illustration of redundancy most often cited is English orthography in which numerous letters are dispensable without destroying the meaning. But perhaps more illuminating are illustrations drawn from telegraphic communication. Often a key word is simply repeated to insure its correct transmission. For identifying the recipient of a telegram, a name and address are usually sufficient, but when we send a substantial sum of money, we often use a question to insure proper identification such as "What was Aunt Edith's maiden name?" This makes an erroneous delivery very much less likely.

In a complex system of elements interrelated by output-input and feedback, not all inputs produce outputs. It is the nature of such a system to prehend information in patterns. Quastler terms this process "feature sampling" to cover such diverse cases as enzyme catalysis, serological reactions and gestalt perception. The prehension depends on positive reaction to some inputs and indifference to others. Output occurs only when the proper combination of inputs has effectively impinged. Now if the effective combination is enforced by the repetition of some signals or if the pattern is presented in more than one way, the chance of the prehender's missing it entirely is much less than if it is ticked off with the minimum of items.

Noise also in information theory is a concept rather different from the ordinary meaning of the word. In telephonic communication adventitious sounds in the receiver, however produced, interfere with reception and reduce the amount of information transmitted. This is the historical origin of the term but it has been generalized to mean any random event of the same nature as a signal which thus changes the pattern of a message and reduces its information content. In any transmission process through which the output of one element becomes the input of another, there is always the possibility that random events not emitted by the sender will appear to the receiver indistinguishable from signals. If a message is relayed again and again, it will gradually lose information and ultimately become completely random. Just as the ratio of amount of information in a message to the minimum necessary to express it determines the redundancy; the ratio between the amount of information sent and the amount received determines the "equivocation," the amount lost because of noise. Therefore, for a given message transmitted over a given channel, if the redundancy is greater than the equivocation, the probability will be high that the message received will be accurate.

V. BIOLOGICAL INFORMATION

To return now to the coding of biological information, what we have in the fertilized egg, formed by the union of two gametes, is the genetic code—the form in which the information is transmitted from generation to generation. There is much still unknown about the nature and functioning of this genetic code, but there is pretty general agreement that DNA plays a very important role in the process. Not only is there overwhelming evidence—ranging from bacterial transformation to the complex body of correlations between chromosome behavior and genetic analysis—that DNA is involved in genetic change, but its structure and properties are ideal for the coding of information. The four different bases can theoretically be arranged so that for a sequence of n nucleotide pairs there are 4^n different possible permutations. Estimates of the number of nucleotide pairs in genetic material vary from 8×10^4 for phage T4 (Benzer, 1957) to 4×10^9 for a human gamete (Muller, 1958). These would yield, respectively, $10^{4.8 \times 10^4}$ and $10^{2.4 \times 10^9}$ possible arrangements. Other possible models of coding may change these estimates substantially. It is certain that to get the number of workable combinations they must be reduced by the requirements of integration as defined by Quastler (1959). But making all such allowances, the number of arrangements available in the DNA of any organism with which we are familiar appears to be many, many times any estimate of the number of its genes.

The gene is the descendent of Mendel's "factor," the segregating unit. With the discovery of linkage it became the unit of recombination—still the particular segregating element. With the development of biochemical genetics an attempt was made to identify the unit of recombination with the unit of physiological function—one gene, one enzyme. But the results of finer genetic analysis have shown that the unit of recombination is very often smaller than the functional unit, and what constitutes the latter becomes determined by the level at which we define function, whether the synthesis of a peptide sequence or the production of a turned-up wing. The gene has become thus somewhat blurred in its outline, but estimates of the numbers have run from "several" in bacteriophage to tens of thousands in man (Dobzhansky, 1955). The estimates for number of nucleotide pairs are greater than these by factors of more than 100; the estimates of the number of possible arrangements per gene, therefore, begin at 4^{100}. At present, in those cases where genetic analysis has been most exhaustive, the gene becomes the cistron, that portion of the genetic map within which different mutants are non-complementary, that is, show the cis-trans effect. Within a cistron there may be two or more recons—units of recombination—and two or more mutons—sites of alteration which produce phenotypic change. Operationally, it is exceedingly difficult to measure the muton separately from the recon.

In phage T4 where this type of analysis has been carried out by Benzer (1955, 1957) with great thoroughness, there are two adjacent cistrons, mutations in both of which affect the host range. Mutations in different cis-

trons are complementary in the trans configuration; those within the same cistron are not. By intercrossing numerous "stable" mutants—47 in cistron A and 24 in cistron B—and getting recombination values, it was possible to map the mutation sites with extreme precision. Benzer concludes that the smallest recon is very close to a sequence of only two nucleotide pairs and that the smallest muton is very close to the same value—not more than five pairs. This is impressive evidence that very slight changes in the genetic code can produce altered phenotypes, but it is no evidence that all changes in the code have phenotypic effects. Although the smallest muton may involve as few as five nucleotide pairs, the cistron has of the order of 4×10^3 pairs! The number of mutational sites within the cistron is about 40; Benzer estimates, on the reasonable assumption that further investigation would uncover others, that the total is "more than 100." This suggests strongly that the number of changes in the genetic code within the cistron which produce alteration of the phenotype is a limited proportion of the total.

The 47 mutants used in mapping cistron A were chosen from among several hundred because they were "stable." Those rejected were "unstable." Either they had a high rate of reversion to wild type or they were "leaky" —expressed the mutant phenotype inconstantly. Such mutants are unsuitable for mapping; one cannot distinguish in the same experiment between recombinations, reversions and capricious variability.

Let us consider these four different states of the cistron—(1) wild type, and (2) stable, (3) leaky, and (4) reverting mutant—from the point of view of information theory. From the wild type state the coded message goes through without fail to produce the wild phenotype. From the stable mutant the message does not get through and the mutant phenotype results. From the leaky mutant the message is sometimes blocked as it should be from a good mutant and sometimes is transmitted—leaks through. Different leakies leak and block in different proportions; some produce only a few non-mutant phenotypes; others approach wild type in their performance. All these differences look like different relations between redundancy and equivocation. The interpretation would be that the wild type cistron is so coded that there is always sufficient redundancy to overcome any noise in the channel. In the stable mutant the coding is so defective that even when noise is at a minimum the message is never transmitted. The leaky mutants are intermediate. In those which approach wild type, equivocation is only occasionally high enough to swamp redundancy and block transmission; in others which leak only slightly equivocation is only occasionally low enough to allow the slight redundancy to overcome it.

Under the same interpretation the reverting mutant resembles the leaky in that the message is sometimes blocked and sometimes gets through, but it differs in that when the message does go through, the change is in the coding rather than in the transmission and is permanent—what we call a genetic change. In these mutants certain random events produce changes in the code which restore it to the wild type condition, change it from an unintelligible message to an effective one. When reversion occurs, an in-

creased redundancy is made permanent. Each reverting mutant has its own probability that such a restorative event will occur. Some independently isolated mutants occupying the same site on the map can be distinguished only by their characteristic probabilities of reversion.

Summarizing our interpretation, the entire sequence of some 4,000 nucleotide pairs comprising the cistron encodes a message which has to do with host range. In the wild cistron the message is so effectively coded that transmission rarely fails. There are many sites in the sequence where changes can alter the efficiency of the coding. Such changes may block the message completely (stable mutant); they may merely make it less effective in overcoming noise (leaky mutant) or they may block the message but leave the code in such a state that it has a greater or lesser probability of being restored to effectiveness. Within the cistron the number of possible permutations of nucleotide pairs is many powers of ten greater than the number of mutants isolated after intensive experimental work. It seems impossible to allay the suspicion that the wild type message must be capable of being coded in a number of different ways with different degrees of redundancy, that wild type cistrons must, therefore, differ in the details of their coding and that the notion that the wild type cistron is coded in a unique way containing the exact amount of information necessary to produce the wild phenotype cannot be a realistic description of the facts.

Entirely analogous evidence for the versatility of the genetic material in coding messages can be found in the results of similar precise genetic analyses made in several other organisms (Hartman, 1957; Pontecorvo, 1958). Nor is the minute analysis of the cistron the only source of evidence for redundancy in the genetic code. Another long known and obvious one is the suppressor gene, a message in a site in one portion of the genome which takes over and does what a mutant at another locus fails to do. When both the suppressor and the wild type allele are present, there must certainly be redundancy.

Similarly, the concept of isoalleles, described by Stern (1943) almost twenty years ago, can clearly be understood in terms of redundancy. Careful measurement of the effect of wild type alleles of different origin in their interaction with mutant alleles at the locus shows that each isolate has slightly different characteristics. This must result from slight differences in the coding of the wild type message in each separately isolated allele. Differences in effectiveness in producing the wild phenotype are most likely correlated with differences in redundancy. The fact that all individually isolated mutants and wild type alleles which have been intensively studied show individual characteristics can scarcely be explained except as reflecting differences in the details of the coding they contain.

VI. INFORMATION, DEVELOPMENTAL EFFICIENCY AND HETEROSIS

To come now, full circle, and return to our hatching ratios, every diploid organism, since it has its genetic material in duplicate—two copies of the code—has, of course, an irreducible level of redundancy. But if the two

copies differ—paraphrase rather than repeat—the effective redundancy may be much greater. The difference in viability commonly observed between random heterozygotes and isogenic individuals is striking evidence of this. Our experimental results seem to point to the assumption that inbreeding, by increasing the homozygosity of the genome, that is, reducing the proportion of loci occupied by alleles of different origin, has greatly reduced the redundancy in the total complement of genetic information. The message is a much more complex one than that of any given allele, but the overall effect is the same. As the redundancy is reduced, transmission is more likely to be impeded by noise and the probability of its successful completion is lessened. The randomly breeding population does not have a perfect code. It always shows a small number of failures, but this small proportion is constant and relatively independent of the environment. The inefficiencies of the inbred codes have their own characteristics. Each has its mean proportion of failures. Variations in these proportions are much greater than can be explained by chance, but those environmental vagaries that produce them in one line are not the ones that produce them in another. Otherwise the variations would be correlated between lines. That there is a correlation between the eggs of two groups of parent flies within one line, shows that the environmental events causing variation in the hatching ratio have acted similarly on the two groups. That there is no such correlation between two groups in another line shows that, there, the events responsible must have been acting differently on the two groups and are in all likelihood different events from those producing the correlation in the other line. The differences in performance between the populations indicate that the inefficiencies of coding differ from population to population.

The hatching ratios of the crosses between Sy and SySP give some interesting evidence of information coded in non-chromosomal sites. The difference in hatching ratio between eggs of Sy and SySP in the cross between the two populations shows that part of the information necessary for successful development must be coded in the non-chromosomal part of the egg. The same genome which is 86 per cent successful in hatching when functioning in an Sy egg is successful in only 50 to 60 per cent of the time in an SySP egg. This is not at all surprising in view of what we know of the complexity of the structure of cytoplasm and of the fact that the information coded in the chromosomes must be worked out through the materials found in the cytoplasm and yolk. That information stored in these non-chromosomal elements is ultimately derived from chromosomal information is strongly indicated by the hatching ratio of the eggs of F_1 females of the SySP × Sy cross fertilized by their own sibs. This ratio is actually higher than that of the Sy population—.9825 in one experiment and .9856 in another. The SySP genome produces eggs of inferior developmental potential, but a female with an SySP-Sy genome developed from such an egg produces eggs even more effective developmentally than those of her Sy grandmother.

The performance of these inbred and randomly breeding populations with respect to hatching ratio is very similar to that found by Gowen (1952) with

respect to egg production. "The hybrids are good," he wrote, "because on the average, all members of the cross are good producers. . . . It is the consistency of high performance which calls for explanation." It seems very likely that the explanation is to be found in the amount of redundancy in the genetic code and that the phenomenon of heterosis is to be explained on this basis. Redundancy functions neither as overdominance nor as the covering of deleterious recessives. Its effectiveness arises from the fact that no two chromosomes of different origin are coded identically and that therefore two of them are bound to contain more information than one. The more information in the genome, the more smoothly development will proceed; the more similar its overall effect will be from individual to individual; and the smaller the variance will be for most characters. The reduction in variance is probably a more general effect of heterosis than increase in size, in yield or in other measurements. In another sense, the effect of redundancy is both the covering of deleterious recessives and overdominance. Each differently coded allele covers some of the cryptic deficiencies of the other; they pool their potentialities to produce all of which they are cooperatively capable.

A species or population of organisms must be conceived as a continuous, integrated system of input-output relations between the various elements of which it is made and its environment. The continuity is cyclical rather than uniform and the information therefore goes through cyclical change (see Schmalhausen, 1960). The information coded in the chromosomes is only part—though a very important part—of the total. The whole system of input-output relations is integrated both internally and externally and the probability of the continuity of the system is its fitness or adaptive value. Since the system is capable of modifying its integration and is constantly doing so, fitness or adaptive value is extremely difficult to define and more difficult to measure. Coded information newly brought together—from different strains or races, for example—producing through development, characters such as increased size, greater longevity or higher fecundity may or may not be adaptive depending on whether the information and the resulting characters can be assimilated into the integrated system both internally and externally. Often they cannot and disappear in subsequent generations as a result of recombination in the formation of F_1 gametes, of F_1 sterility or in many other possible ways. This is the situation that Dobzhansky (1952) has termed luxuriance. Sometimes interspecific or interstrain crosses bring together such different information that it is not sufficiently integrated for successful development. There may be no offspring produced; there may be very few; what there are may be abnormal. To be adaptive, information must be assimilated into all phases of the cycle of the organism—genetic, epigenetic, behavioral and ecological.

The evidence respecting redundancy in the genetic code is strongest for its existence between homologous loci along the chromosome, but this is clearly not the whole story. As we have seen, it can also exist in the composition and organization of the non-chromosomal portion of the egg. There cannot be many cases where the redundancy found in the chromosomes is in

the form of simple linear repetition, for anything beyond a very small deletion is almost always inviable in haploid or homozygous condition. Nevertheless, there is evidence that redundancy may inhere in the whole chromosome as a unit. This must be the reason for the greater viability and more efficient development associated with intact as distinct from derived chromosomes in interpopulation crosses (Wallace, 1954) and for the non-random association of separable chromosomal inversions found in wild populations (Levitan, 1958). Here we see evidence of a sort of epistatic—non-homologous—redundancy, in fact a cis-trans effect for whole chromosomes. The structural and functional peculiarities of chromosomes such as the Drosophila X which are facultatively hemizygous (Dobzhansky, 1957) strongly suggest special methods of coding, very likely to safeguard against dangerously low levels of redundancy.

A concept which acquires deeper meaning in the light of the role of redundancy as suggested here is that of the phenodeviant (Lerner, 1954). This is a character which turns up persistently in inbred lines, which cannot be assigned to one or a few segregating loci and which disappears on outcrossing. Lerner attributes it to a lowering of general heterozygosity below a threshhold. Suppose that in the integrated system of a given organism there is a point of low insurance against leaving the creode—a sharp curve or a precipitous drop beside the highway in the epigenetic landscape. A weakness of this sort, just as well as some positive character, may result from a polygenic configuration. What insurance there is against it will lie in redundancy distributed through the polygenic system. A general lowering of redundancy will often uncover it but the character itself will not segregate with a recognizable pattern. Raising the redundancy by outcrossing will cause it to disappear.

Lerner's concept has been called mystical (Morton, 1960). Is this explanation mystical? In one sense, perhaps, in the sense that there is something mystical about all generalizations. There were mystical overtones in Eddington's (1928) pointing out that while entropy could associate with mass and distance because it spoke the language of arithmetic, it could also hobnob with beauty and melody because, like them, it was arrangement. Weaver (Shannon and Weaver, 1949) carried the figure on by saying that entropy as the foundation of information was intimately related to meaning; it spoke also the language of language. Now it appears that entropy engenders the pulse of life, not merely the obvious manifestation like the beating of the he art, but but the protean on-and-off complexity of input-output which lies behind the click of the telegraph, the brightening and fading of the dots on the television screen, the call of the spring peeper and the modulation of the human voice. It speaks even the language of love. *Ex nihilo nihil fit?*

SUMMARY

Measurements of the ratio of hatched eggs to total in randomly breeding and inbred populations of *Drosophila melanogaster* show that the two differ

strikingly and consistently. In the former the ratio is high and its variance low; in the latter the situation is reversed. The inbred embryo has a low and variable probability of developmental success even in a very constant environment. Recent attempts to apply information theory and cybernetics to biological phenomena suggest a new way of looking at these facts employing the concepts of redundancy, noise and equivocation with respect to developmental efficiency. Biological information is passed from one generation to another coded in the genetic material. It is generally agreed that DNA plays a major role in this coding and there is very good evidence that very slight changes in nucleotide sequences can produce phenotypic change. But there is also excellent evidence that the number of possible permutations of the DNA is far, far greater than the minimum necessary for coding the genetic information and that actually the information is coded in numerous different ways with different degrees of redundancy. It is suggested that the low and variable hatching ratio of the inbred population is the result of a lowering of redundancy in a genome with more loci occupied by identical alleles. The data concerning hatching ratio exactly parallel those for heterotic characters generally and it appears likely that redundancy in the genetic code may be a general explanation of heterosis more satisfactory than either overdominance or the covering of deleterious recessives.

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A DEVELOPMENTAL STUDY OF RECESSIVE LETHALS FROM WILD POPULATIONS OF DROSOPHILA MELANOGASTER

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Various investigations (see review by Spencer, 1947; Paik, 1960) have shown that a great store of variability, concealed in the heterozygous condition, exists in wild populations of Drosophilae. Of the many types of "mutants" which occur in natural populations, those which affect the viability of the carrier are of special interest. Mayr (1955) has pointed out that the adaptive value or fitness of the genotype must ultimately be studied in terms of developmental and physiological genetics. Most of the earlier work on recessive lethals from wild and laboratory populations has been statistical in nature and only recently have there appeared investigations dealing directly with developmental aspects (Rizki, 1952; Wallace, 1952; Seto, 1954; Milkman, 1960). Rizki studied the ontogenetic distribution of lethality in naturally occurring recessive lethals of *Drosophila willistoni* and the author began a similar study with *D. melanogaster.* Although the results reported were similar in the two species, more information of a comparative nature would be useful. The present study was conducted to determine if the ontogenetic distribution of lethality of naturally occurring lethals is the same from place to place and from time to time. A supplementary investigation was made on a population of "wild" Drosophilae maintained in the laboratory. By comparing the relative period of actions observed in these populations with that reported in a group of lethals which occurred spontaneously in the laboratory, it may be possible to judge whether or not natural selection acts differentially on lethals which produce their lethal effects at different times in development.

MATERIALS AND METHODS

The general plan of the investigation is schematically outlined in figure 1. Toward the end of the summer of 1959 the first collections of Drosophila were made by setting out baited traps in clumps of apple trees at three separate sites near the outskirts of the Berea College campus. On favorable days, collections were made every other day and at no time were traps left out longer than a week. Males from the collections were mated immediately to females of the special strain used in the *Sifter* mating scheme (Muller, 1951) to isolate second chromosome recessive mutants. The females were placed individually in vials and from the progeny of each female a single male was selected at random for mating. Any *Sifter* mating which produced at least two wild type males and as many or more wild type females was considered non-lethal and discarded. Vial cultures which did not produce wild type flies were examined again the next generation and spot-

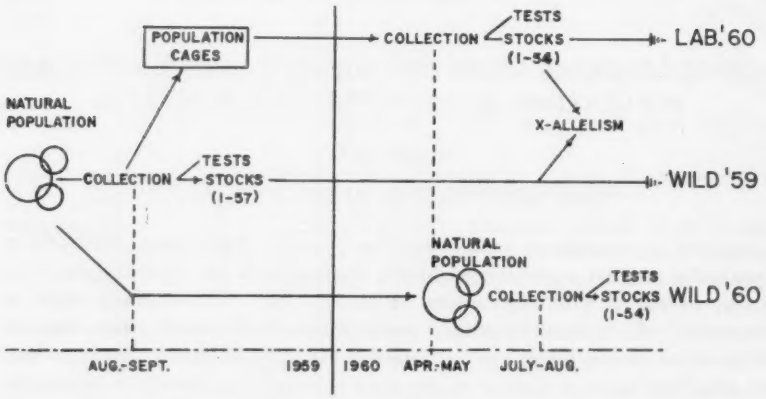

FIGURE 1. Schematic representation of the plan of investigation. Two samples of wild *Drosophila melanogaster* were collected on successive years (1959 and 1960) from natural population and a third sample from a laboratory maintained population of wild flies. These samples were tested for their recessive lethal content, for allelism rate and time of lethal action of the lethal factors.

checked thereafter. No attempts were made to classify the chromosomes as lethals, semi-lethals, subvitals and normals. The chromosomes containing the recessive lethals were maintained in a balanced condition with *Cy* for further tests. The recessive lethal strains collected from wild populations during the summer of 1959 are designated as the *Wild* 1959 series in this study.

The surplus unselected males and females from the 1959 field collections were used to start the laboratory population. Many of the males used in the *Sifter* matings were also subsequently added to three population cages, each containing ten slanted food vials, which altogether could support a total population of about 1500 to 3000 flies. During mid-winter heavy soil nematode infestations in the population cage food vials reduced the population to less than one-third its normal adult number but with special care the population recovered its normal vigor. As the population increased, additional population cages were started as a precaution and at the end of an eight-month period, there were five healthy cages in all from which random samples were taken to isolate lethal second chromosomes. The lethal strains of this collection are designated as the *Laboratory* 1960 *series.*

Early in the summer of 1960, another collection of wild Drosophila was made from the same sites as in the previous year. The recessive lethals from this collection make up the *Wild* 1960 series. An intrapopulation allelism test was made for each group of lethals and a further cross allelism test was performed between lethal chromosomes of *Wild* 1959 and *Laboratory* 1960. Subsequently all of the lethals were examined for their developmental stage of lethal action and an ontogenetic distribution was compiled for each series of lethal chromosomes.

Allelism tests

To determine the number of independent lethal factors present in each collection group, the various *Cy/le* balanced strains were crossed with one another. Any mating of two lethals which produced many wild type flies among the progeny was considered non-allelic. In this manner a total of 57 lethal lines of the *Wild* 1959 series, 54 of the *Wild* 1960 series, and 54 of the *Laboratory* 1960 series were tested. An interpopulation test of independence was made between 48 different *Cy/le* strains of *Wild* 1959 and 30 different *Cy/le* strains of *Laboratory* 1960. Since these two populations have a common origin, the cross tests should give some indication of the degree of independence of the lethals in the two series.

Determination of the stage of developmental action

The stage of developmental action of each lethal was roughly determined by a method essentially like that used in a previous study (Seto, 1954). Each *Cy/le* strain was crossed to *cn/cn (cinnabar)*, and from the F_1 offspring the *non-Curly* lethal heterozygotes were mated *inter se*. Twenty-five per cent of the offspring from this mating are expected to be of the *le/le* homozygous type and will die at some stage in the life cycle. Before the counts were begun, the parent flies (F_1 *non-Curly*) were aged for a few days and the first few batches of eggs were discarded. Counts then were made of the number of eggs laid, the number which hatched, the number of pupae formed, and the number of adults which emerged. Generally counts from six to eight replicate vials were totaled to obtain the final figures. The particular stage or two consecutive stages, which displayed the expected amount of mortality was designated as the developmental period of lethality. Very few of the lethals tested were multi-phasic in their developmental effects. The various lethals were then classified as egg lethals (E), egg-larval boundary lethals (E/L), larval lethals (L), larval-pupal boundary lethals (L/P) and pupal lethals (P). For each group of lethal chromosomes, a frequency distribution was made of the number of lethals which expressed their effects at different stages of development.

RESULTS

The frequency and per cent of lethal chromosomes of alleles among the lethal chromosomes are given in table 1. Although all possible combinations

TABLE 1

The frequency and per cent of lethal chromosomes and of alleles*

Source	Chromosomes	Lethals	Per cent	Cross-tests	Alleles*	Per cent
1. *Wild* 1959	350	110	31.4	1586	15	0.9
2. *Wild* 1960	345	129	37.4	1418	34	2.4
3. *Laboratory* 1960	236	55	23.4	1403	43	3.1
4. 1 × 3	...	...	...	1440	4	0.3

*Refers to *Cy/le* crosstests which failed to produce wild-type flies.

TABLE 2

Frequency of appearance of allelic lethal chromosomes

Source	No. of lethals tested	Frequency of appearance							No. of different lethals
		1	2	3	4	5	6	7	
Wild 1959	57	41	3	2	1	0	0	0	47
Wild 1960	54	34	3	1	1	0	0	1	40
Laboratory 1960	52*	15	8	1	2	2	0	0	28

*Although 54 lethals tested, results on two incomplete and were not included here

were attempted in the *Cy/le* crosstests, some of the matings were not successful. Many of these matings and others which failed to produce wild-type flies were retested. The per cent allelism was then computed as the ratio of matings which did not produce wild-type flies to the total number of successful crosstests. The frequency of allelic chromosomes for lethals in the different series is given in table 2. A comparison of the distributions with relative frequencies calculated by means of the Poisson series indicated that the *Wild* 1959 and *Wild* 1960 lethal series are probably random with respect to frequency of appearance of the different lethal factors. It appears, however, that the *Laboratory* 1960 distribution is significantly different. Two other lethal chromosomes in the laboratory series and two more in the *Wild* 1960 series were incompletely tested for allelism and were not included

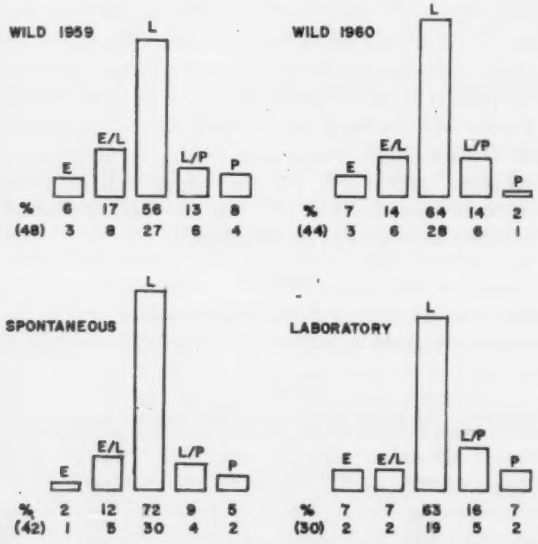

FIGURE 2. The ontogenetic distribution of lethality of lethals from various sources. In each sample of second chromosome recessive lethals, the number and percentage of lethals which have their effects at a specific stage in development are given.

TABLE 3

Comparison of the ontogenetic distributions of lethality of recessive lethals from various sources

Source	Number in each stage					Total‡
	E	E/L	L	L/P	P	
Wild 1959	3	8	27	6	4	48
Wild 1960	3	6	28	6	1	44
Laboratory 1960	2	2	19	5	2	30
Madison 1951*	3	10	16	6	8	43
Spontaneous†	1	5	30	4	2	42

Results of Chi-square test:

$\chi^2 = 17.221$ d.f. 16 $P = .38$

*Seto (1954).

†Combined data from Hadorn (1952), Rizki (1952) and Seto (1954).

‡Numbers may not agree with table 2 totals since additional lethals were included in this study.

in the summary. These were tested for their developmental time of action and included in the ontogenetic study.

The results of the time of action determination are summarized graphically in figure 2 and table 3. In the graph each bar represents the relative number of independent lethals that caused developmental arrest at a particular stage in the life cycle. Below each bar are given the actual number and percentage of lethal factors in the category. In addition to the new data on the wild and laboratory populations, a frequency distribution of spontaneously occurring lethals, compiled from several sources, has been included for comparison. The numerical values of the different ontogenetic distributions have been compared and the results of a χ^2 heterogeneity analysis are given in table 3. All of the distributions appear to be alike. The statistical analysis (5×5 contingency table) indicates no significant difference among the various populations. The data are a good fit to the assumption that the ontogenetic distributions of lethality displayed by the lethals from various population origin are basically alike.

DISCUSSION

Frequency of recessive lethals

The frequencies of 31.4 per cent and 37.4 per cent and the allelism rates of 0.9 per cent and 2.4 per cent for recessive second chromosome lethals in the wild populations under study are similar to values reported by Ives (1945, 1954), Band et al. (1959) and Hiraizumi and Crow (1960) for several North American populations of *Drosophila melanogaster* and by Goldschmidt et al. (1955) for Israeli populations of the same species. Lethal frequencies of this magnitude have been found in other species by Pavan and Knapp (1954) and others. Still other studies of lethal frequencies have revealed geographical differences in concentration of lethals. Lower rates have been

reported by Dubinin (1946) for Russian populations, by Oshima and Kitagawa (1961) for Japanese populations and exceptionally low frequencies of 7.5 to 14.9 per cent were found in Korean populations by Paik (1960). Paik explains the low incidence he observed as the consequence of small breeding units of Drosophila at the collecting sites. Seasonal fluctuations in lethal frequencies have also been reported (Dubinin, 1946, Band et al., 1959; Crow, personal communication), although this has not been ascertained by others (Ives, 1954; Goldschmidt, 1955). The values of 31.4 per cent from the *Wild* 1959 collection made in September and 37.4 per cent from the *Wild* 1960 sample obtained in July are probably not significantly different.

If the lethal frequency of 23.4 per cent and the allelism rate of 3.1 per cent in the laboratory population are not sampling errors, they would represent a reduction of about one-quarter from the incidence of lethals in the source population (*Wild* 1959) and a threefold increase in allelism. A similar proportionate decrease in lethal concentration and change in allelism rate have been observed by Ives (1945) in a Maine population of wild Drosophila that had been maintained in the laboratory for 60 generations of mass matings. This reduction in lethal frequency and increase in both allelism rate and frequency of appearance of allelic chromosomes have been interpreted as the consequence of decrease in population size and subsequent increase in inbreeding. This explanation is especially applicable in this study since a population bottleneck occurred during the nematode infestation of the population cages. The allelism rate of 0.3 per cent between *Wild* 1959 and *Laboratory* 1960 is similar in magnitude to values obtained by others for interpopulation allelism rates. Values as low as this have also been observed in laboratory control populations (Ives, 1945) and among lethals newly induced by irradiation (Wallace, 1950) and probably indicate independent occurrence of lethals in a population.

The literature on the incidence of lethals in natural populations of Drosophila has been sufficiently reviewed by Dubinin (1946), Spencer (1947) and Pavan and Knapp (1954). Since the data are similar to that obtained by Ives (1945, 1954) and he has discussed the genetic structure of wild populations of *D. melanogaster* in North America, I shall not attempt to speculate on the breeding structure of the Berea wild populations.

Ontogenetic distributions of lethality

The comparison of the several ontogenetic distributions (see figure 2 and table 3) indicated that there is no significant difference in the relative proportion of lethals with different ontogenetic effects in the various populations studied. Moreover, the distribution of lethality is similar to that exhibited by spontaneous laboratory lethals. Since it is probably safe to assume that the occurrence of spontaneous lethals is random, the similarity of the other population distributions to it would suggest that there is little, if any, evidence in this study of a differential selection of recessive lethals with respect to time of developmental effect. Thus, it appears that neither season (early summer vs. late summer in Berea) nor geographical area

(Berea, Ky. vs. Madison, Wis.) nor mode of culture (natural vs. laboratory population conditions) have any striking effect on the kind of recessive lethals which survive heterozygously.

An examination of the various ontogenetic distributions shows that there is a predominance of larval lethals. One might at first glance interpret this as suggesting simply that there are more genes which act during the larval period than at other times or that this stage is especially sensitive to developmental disturbances. Such an interpretation is perhaps an oversimplification. The larval stage is not necessarily a period of unusually high developmental activity but one in which the already well differentiated larval organ systems increase in size by cell growth. Cell division at this stage appears to be limited mainly to the imaginal discs and rest cells (Bodenstein, 1950). Moreover, others who have studied larval lethality in more detail (Hadorn, 1951, 1952, 1955; Rizki, 1952; Oster, 1952, 1954) have found that practically all larval deaths occur either in the first or the third instar. Mortality in the first larval instar may be the consequence of a delayed expression of embryonic abnormalities and/or failures in the functional system which would normally be expressed at hatching. The cessation of development during the third instar is probably an early manifestation of metamorphic disturbances which usually occur at the larval-pupal boundary.

SUMMARY

Samples of *Drosophila melanogaster* from three populations of common origin were examined for their load of recessive lethals. Two of the samples were collected from wild populations in Berea, Kentucky, on successive years and a third sample was obtained from a laboratory population, initiated with flies from a wild population and maintained for an eight month period. These samples were tested for frequency of second chromosome recessive lethals, for allelism rate and for ontogenetic distribution of lethality of the lethal factors. The frequency of recessive lethals in the samples from wild populations in 1959 and 1960 were 31.4 per cent and 37.4 per cent, respectively, and that of the laboratory population was 23.4 per cent. The allelism rates for the lethal factors in the three populations were 0.9 per cent, 2.4 per cent and 3.1 per cent, respectively.

The ontogenetic distributions of lethal action of the mutants in the three Berea samples, in an earlier sample from a Wisconsin wild population, and in a group of spontaneous lethals were not significantly different, each with a large majority (56 to 72 per cent) which were effective in the larval stage. This suggests strongly that the effects (or lack of effect) of natural selection are the same in all of these ontogenetically different types of lethals.

It appears that the genetic variability, as measured by the recessive lethal frequency, allelism rate and ontogenetic distribution of lethality of the lethals, is about the same from year to year in the areas studied. When a sample of wild population was transposed into population cages, there appeared to be an overall decrease in genetic variability.

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ON FOURTH CHROMOSOME LETHALS FROM A NATURAL POPULATION OF *DROSOPHILA MELANOGASTER*

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The fourth chromosome in *Drosophila melanogaster* is unlike the two other autosomes of this species in several respects. Most obvious among these disparities is the size differential. At somatic metaphase, chromosome four is a dot-like microchromosome of less than 0.3 micron compared to the second and third chromosomes, each of which approximates three microns in length (Cooper, 1950). Of the more than 5,000 bands distinguished by Bridges (1942) in the giant chromosomes of larval salivary glands, only 50 were identified in chromosome four. The larger autosomes by contrast contain about 2,000 bands apiece.

Two additional major dissimilarities set the small autosome apart from the other members of the complement. In cytologically normal diploid females, crossing over between fourth chromosome homologues does not occur at a detectable frequency, if at all. This is clearly different from the considerable genetic exchange taking place within the other chromosome pairs. Worthy of mention also is the fact that zygotes carrying inequalities in the number of second and third chromosomes die while chromosome four monosomics and trisomics survive, at least under laboratory conditions.

These properties of the fourth chromosome render it inaccessible for certain types of genetic investigation. Frequently, a belief that the effect of the microchromosome will be negligible prompts workers to ignore it in their research. On the other hand, the viability of haplo-IV and triplo-IV flies can be exploited in gene dosage studies (Schultz, 1935; Stern, 1948, and earlier).

The significance of chromosome four variability at the population level is of primary interest to the author. Earlier work (Hochman, 1961) has dealt with relative adaptive values of wild-type isoalleles in this autosome. The present investigation concerns the first instance of fourth chromosome lethals detected in a natural population. Although preliminary in nature, this report indicates that large-scale testing of fourth chromosomes from wild populations, similar to that conducted by Ives (1945, 1954) on naturally occurring second chromosome lethals, is both feasible and potentially valuable.

MATERIALS AND METHODS

On October 19, 1960, adult flies were collected on decaying apples and grapes in Wallace's orchard on U. S. Highway 25W near Powell, Tennessee.

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Two species, *D. melanogaster* and *D. bydei* were abundant and over 100 individuals of each species entered the traps. Also captured were some 20 *D. simulans* and about ten other flies not specifically identified.

The trapped *D. melanogaster* were tested for the presence of fourth chromosome recessive lethals. Three generations of simple crosses are required to uncover such lethal genes. In the initial crosses wild males (either those caught in nature or sons of captured females, which had been placed separately in food vials) were mated to females containing a dominant marker in each of their fourth chromosomes. Phenotypically these females have small, thin bristles and reduced eyes owing to the Minute-4 deficiency (*M*-4) and the eyeless-Dominant duplication (ey^D), respectively. A single $ey^D/+$ son from each mating was backcrossed to the *M*-4/ey^D strain and the second generation $ey^D/+$ flies were intercrossed. The absence of wild type, that is, non-eyeless-Dominant, individuals among the third generation progeny in any line discloses that a particular wild chromosome is lethal when homozygous. It is reasoned that a lethal gene found through this procedure was carried, with virtual certainty, by a member of the natural population sampled. There is, of course, an extremely small chance that a lethal mutation could have originated in a sperm cell during the initial *M*-4/$ey^D \times +/+$ mating.

The foregoing crossing scheme establishes any chromosome four lethal in a balanced lethal stock since the ey^D/ey^D genotype is inviable and no recombination takes place.

Lethal genes derived from the Powell population were cross-tested for allelism *inter se* and with seven non-allelic fourth chromosome lethals of X-ray origin generously provided by Dr. M. M. Green of the University of California at Davis.

The possibility that the lethals from nature might interact with some of the known fourth chromosome dominant "visibles" (which are also lethal when homozygous) was examined. In addition to the previously described strain, *M*-4/ey^D, two stocks, bent-Dominant/cubitus-interruptus-Dominant (bt^D/ci^D) and sparkling-Cataract/Cell-2 (spa^{Cat}/Ce^2) furnished the means of evincing such interactions. Individuals heterozygous for the Powell lethals ($ey^D/1$) were mated with bt^D/ci^D and spa^{Cat}/Ce^2 flies. In each cross the offspring lacking the ey^D duplication are expected to fall equally into two classes. The absence of any phenotypic category indicates a lethal interaction between the visible responsible for the phenotype and the lethal being analyzed.

In a similar manner, possible visible interactions between the lethals and most of the known recessive genes on the fourth chromosome were investigated. The recessives studied were cubitus-interruptus (*ci*), grooveless (*gvl*), eyeless-Russian (ey^R), shaven-naked (sv^n), bent (*bt*), abdomen rotatum (*ar*), sparkling (*spa*), and poliert (*pol*). With the exception of the last-named, all of these mutants, dominant and recessive, are described in Bridges and Brehme (1944). Poliert, discovered by Rickenbacher (1954), affects eye size and texture and behaves like an allele of sparkling.

It should be emphasized at this point that the nature of the microchromosome precludes the localization of new mutants by the usual recombination methods. Instead, one can resort to the interaction tests detailed above. An interaction between a previously unplaced lethal and one of the mapped genes suggests an allelic relationship. A map of chromosome four based on crossing over in diplo-IV triploid females (Sturtevant, 1951) and on pseudodominance effects caused by deficiencies (Fung and Stern, 1951) was utilized. Representatives from all but two of the known loci on the microchromosome were tested against the Powell lethals. All crosses were maintained at 25 ± 2°C except those involving *bt*, which were raised at 30°C to enhance the expression of this gene.

Finally, genetic tests were conducted to determine if the presumed chromosome four lethals are in actuality second or third chromosome lethals that are linked by translocations to the microchromosome. (The method used to derive the lethals eliminated any chance that they might represent 1;4 exchanges.) In the translocation test, females homozygous for recessive markers on the three autosomes were mated to males containing a Powell lethal balanced over ey^D. Normal-eyed sons from this cross, heterozygous for the markers, were backcrossed to the multiply-marked females and a 2;4 or 3;4 translocation would have resulted in progeny devoid of certain of the expected eight phenotypic classes.

RESULTS

Three lethal-bearing fourth chromosomes were found among 104 chromosomes extracted from the wild population studied. No attempt was made to classify the other chromosomes into such viability categories as semi-lethal, deleterious, etc. Only a chromosome that allowed no homozygous individuals to survive was designated as lethal. One of these lethals appeared in a line initiated from a captured male (out of 45 independent male-originated lines), and two other lethals were discovered in different lines derived from 59 females trapped with the males. The lethals were assigned the symbols 1(4)PT-1, 1(4)PT-2, and 1(4)PT-3 to emphasize the fact of their extraction from the natural population at Powell.

Cross-tests of the lethals demonstrated that they are all non-allelic. Tests with the seven X-ray induced lethals revealed two examples of allelism, 1(4)PT-1/1(4)6f and 1(4)PT-3/1(4)4d proving to be inviable combinations. The third Powell lethal, 1(4)PT-2, was not identical with any other and it alone interacted lethally with one of the dominant visibles studied. From the cross $ci^D/bt^D \times$ 1(4)PT-2/ey^D, no non-ci^D individuals appeared among more than 200 non-ey^D flies examined, definitely establishing the inviability of the bt^D/1(4)PT-2 genotype. The simplest interpretation of this result is that 1(4)PT-2 is allelic to, or deficient for, the bt^D locus, or section, of the microchromosome.

Heterozygous combinations of the three lethals and the eight recessive visibles were all fully viable and phenotypically wild type. The failure to

TABLE 1

Allelism tests of three non-allelic fourth chromosome recessive lethals, derived from a natural population in Powell, Tennessee, with various chromosome four mutants. A minus sign indicates that the chromosomal combination dies; a plus sign signifies that the heterozygote is viable, and, in the case of the recessive visibles, phenotypically normal.

Recessive lethals from nature	Induced lethals*			Dominant visibles						Recessive visibles							
	1(4)6f	1(4)4d	5 others	M-4	*ci*^{D}	*Ce*^{2}	*bt*^{D}	*ey*^{D}	*spa*^{Cat}	*ci*	*ar*	*gvl*	*bt*	*ey*^{R}	*sv*^{a}	*spa*	*pol*
1(4)PT-1	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1(4)PT-2	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
1(4)PT-3	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*Green and Gloor, unpublished observations.

uncover any examples of pseudodominance implies that none of the lethals represents deficiencies for any of the loci in which the tested recessive genes are situated. These results, summarized in table 1, serve to localize only 1(4)PT-2 and leave the other lethals unmapped.

The findings in the translocation tests were negative. All expected phenotypic classes appeared, and in the absence of contradictory evidence it is concluded that fourth chromosome factors alone are responsible for the Powell lethals.

DISCUSSION

Recessive lethals have been found on the large autosomes in wild populations of several species of Drosophila. The earliest reports are those of Dubinin (1931) concerning the second chromosome of *D. melanogaster*, and Sturtevant (1937) on chromosomes two and three in *D. pseudoobscura*. More recently, a number of workers have utilized this form of variability to increase our knowledge of the genetics of natural populations. Ives (1945, 1954) demonstrated the existence of geographical and temporal differences in the frequencies of lethal-bearing second chromosomes in *D. melanogaster* populations. Evidence that altered environmental conditions may affect the proportion of these lethals has been presented by Band and Ives (1961).

This examination of chromosome four from nature was undertaken in an attempt to discern the suitability of the microchromosome for studies similar to those mentioned above. Certain properties unique to this small autosome are cited in the introductory section. Their net effect on the occurrence and maintenance of recessive lethal genes on the microchromosome is not yet understood. Judging from the size-factor alone, it would appear inadvisable to expect lethal chromosomes in numbers sufficient to make interpopulational comparisons practicable. Based on Bridges' (1942) counts, the ratio of salivary chromosome bands in chromosomes four and two, respectively, is 1:38. This ratio becomes somewhat less disproportionate if one includes the few bands located in the minute left arm of the microchromosome (see Slizynski, 1944). There is now some evidence that the ratio of lethals in the two auto-

somes in a wild population is closer to 1:10, a value in accordance with the relative sizes of the autosomes at somatic metaphase. The percentage of chromosome four lethals reported here is roughly 3. A simultaneous analysis of the Powell population detected 27 per cent (36 of 132 tested) completely lethal second chromosomes (Lopushinsky, 1961). These latter findings, in addition to their comparative value with respect to the fourth chromosome data, indicate that the natural population sampled is "typical," that is, the measured chromosome two lethal frequency is consistent with the north-south gradient ascertained by Ives (1945).

Although the data are not extensive, they suggest the testable hypothesis that, within a natural population, a chromosome four locus is more likely to contain a lethal than one in the larger second chromosome. Various explanations for this presumed difference include higher lethal mutation rates and/or lowered selection against these extremely detrimental genes in the smaller autosome. Not to be overlooked is the possibility that an inaccurate evaluation of the relative number of loci present in the two chromosomes is obtained when their salivary band totals are compared. Despite the IV:II band ratio and the paucity of visible mutants identified to date on the microchromosome, there may be proportionately more loci capable of lethal expression here than on chromosome two.

Regarding this last point, Ambellan and Prout (1956) calculated, from their observation that 13 radiation-induced lethals constituted only five allelic sets, that the probability is very low that more than 20 loci capable of mutating to lethality are situated in the fourth chromosome. The results given here tend to confirm the Ambellan-Prout estimate. Each of the three Powell lethals proved allelic to a previously known fourth chromosome factor, two to X-ray induced lethals and one to a visible, bt^{D}. (The failure to detect any of the Powell lethals more than once may have been a consequence of small sample and large population sizes.) Presently available evidence therefore suggests a low number of "potentially lethal" sites in chromosome four. However, an upward revision of the Ambellan-Prout figure may be necessitated by recent work of M. M. Green and H. Gloor (personal communication). They induced, with X-rays, 36 fourth chromosome lethals which they believe to be distributed among at least 18 different loci, including nine manifesting no interactions with previously known dominant visibles.

It would be premature to discuss the possibility that selective forces, rather than mutation pressure, might be responsible for a higher than expected proportion of lethal fourth chromosomes in wild populations. Data corroborating the latter assumption must first be collected. Nevertheless, one should recognize that, in addition to size and recombinational differences, characteristics of evolutionary significance may distinguish the microchromosome from the other autosomes. Pertinent here is the supposition by several workers (most recently Krivshenko, 1959) that chromosome four was originally part of the X chromosome. This postulation is supported by (1) the presence of "femaleness-determining" factors on the microchromo-

some (Fung and Gowen, 1960), (2) the discovery of three loci on the X chromosome of *D. busckii* (which lacks a dot chromosome) that are presumably homologous to fourth chromosome genes in *D. melanogaster* (Krivshenko, 1959), and (3) certain X-IV pairing peculiarities (Sandler and Novitski, 1956, and others). None of these arguments are especially convincing, however, and White (1945) thinks it much more likely that a chromosomal complement comprising an acrocentric X and a dot is primitive, and the fusion of these two secondary, in the subgenus (*Sophophora*) which includes *D. melanogaster*.

Now that the feasibility of examining fourth chromosomes on an enlarged scale has been made apparent, one should be able to determine whether or not the microchromosome is merely a miniature edition of the larger autosomes insofar as concealed genetic variability in wild populations is concerned. In order to achieve more meaningful comparisons with the second chromosome findings, subsequent fourth chromosomes extracted from nature will be classified over the whole viability spectrum, instead of the oversimplified lethal-versus-viable categorization. To counteract, in part, those properties of chromosome four that impose difficulties in the localization of new lethal mutations, future studies will include salivary chromosome inspections in addition to the mapping techniques described earlier. Lastly, some useful information may be gained from laboratory cage experiments involving lethal and non-lethal microchromosomes obtained from the same natural population.

SUMMARY

An analysis of 104 fourth chromosomes removed from a wild population of *D. melanogaster* near Powell, Tennessee, revealed three containing recessive lethal genes. These microchromosomal lethals are all non-allelic. Their inability to produce pseudodominance effects with any of the known recessive visibles on chromosome four makes it unlikely that they are deficiencies for these loci. Two of the Powell lethals proved identical to two different lethals of X-ray origin, and a third interacted lethally with bent-Dominant, one of the six dominant visibles tested. These observations support the idea that there are very few sites in the small autosome capable of harboring a lethal allele.

Although the data are not extensive, they suggest that microchromosomally based lethals may be carried in gene pools of natural populations at a frequency higher than one would expect considering the paucity of loci in this autosome. Future investigations, enlarged in scope and improved methodologically, will be undertaken to test this hypothesis.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

SHORT-TERM CULTURE OF LEUCOCYTES FROM MONKEY PERIPHERAL BLOOD*

The short-term culture technique of Hungerford, Donnelly, Nowell and Beck (1959), modified by Beçak (1961), has been effectively used for the study of human karyotypes in our laboratory. In an attempt to find a good and simplified method for the study of karyotypes of primates, we used the same technique for the Rhesus monkey (*Macaca mulatta*). The cultures pre-

FIGURE 1. Karyotype from short-term culture leucocytes, from a male Rhesus monkey (*Macaca mulatta*), showing 42 chromosomes.

*This work was supported by a grant from the Instituto Butantan Research Fund.

TABLE 1

Chromosome counts of the peripheral blood leucocytes from a male Rhesus monkey (*Macaca mulatta*).

	Chromosome number					
	40	41	42	43	44	Total
Cells counted	...	2	22	...	1	25

sented a high mitosis index with perfectly distinguishable karyotypes (figure 1). The results, presented in table 1, show that the normal number of chromosomes of *Macaca mulatta* is 42. This is in agreement with countings previously made by Darlington and Haque (1955) in spermatogoniae and by Chu and Giles (1957) and by Rothfels and Siminovitch (1958) in kidney cells cultivated *in vitro*.

The present technique has the advantage of not injuring the animal. It probably will be useful for extensive karyological studies of the primates aiming to clarify taxonomic and evolutionary problems.

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DIFFERENTIAL NON-GENETIC VARIABILITY IN THE EXPRESSION OF MAJOR GENES AND POLYGENES

An important attribute of polygenes governing the inheritance of quantitative characters is generally taken to be their relatively high instability in expression resulting from their non-genetic variance being as large as their individual genetic effects. A large variance would also occur for a group of polygenes due to the additive property of their variances. This characteristic of polygenes was emphasized among others by Mather (1949), according to whom the "contributions of individual units in a polygenic system are "readily mimicked by non-heritable agencies." Such a viewpoint clearly implies that the major genes (or oligogenes, in Mather's terminology) are less susceptible to environmental variation than the polygenes. However, since there are at present no *a priori* grounds to suggest that the nature and organization of the genetic material in the two gene classes are basically different, it should be of interest to consider a possible explanation for the apparent differences in the amount of non-genetic variability of major genes and polygenes.

Polygenes, by definition, are numerous and have small, additive and dosage effects. Now, if no basic distinction with respect to the genetic material is assumed, it seems reasonable to consider polygenes to be comparable to the hypomorphic recessives which, too, act additively and show dosage effects. Following this analogy between the polygenes and hypomorphs, any genetic or biochemical explanation for differential non-heritable variation in the expression of the wild type allele and its recessive at a locus would hold good for the polygenes and the so-called 'switch' genes. The hypomorphs produce, even in homozygous condition, quantitatively less gene product than the corresponding wild type allele in a single dose and all variation in the quantity of this primary gene product and in its reaction with the available substrate would determine the amount of phenotypic variation in each case. The dominant allele being overactive in the sense that it is haplo-sufficient produces enough or even more gene product than is necessary to react with the whole of available substrate so that the quantity of substrate would limit the range of variation to the relatively lower phenotypic values. In the case of hypomorphs, however, it is the substrate that may be in excess in relation to the quantities of primary gene product, and thus, there would be scope for the entire variation in the primary gene activity to be expressed at the phenotypic level. Accordingly, the distribution of phenotypic values might look skewed or one-tailed in one case and two-tailed in the other. It may be emphasized that on this interpretation, the variance at the level of primary gene action may be of the same order in the case of wild type and its hypomorphic recessive alleles but they may yet show differential phenotypic variability. Another point to note is that while the substrate limit visualized above tends to be less effective in the presence of a large number of hypomorphs or, by analogy, polygenes, competition among the different genetic units for the substrate might contribute significantly to an enhanced variance in such cases.

On the above model the variances due to environment are expected to be greater for the polygenes than the major genes. Experimentally, this expectation may be difficult to verify. However, an analogous comparison can be made between the variances for the wild type allele and its hypomorphs. The extensive studies of Nolte (1952, 1959a, b) on the eye pigmentation in *Drosophila melanogaster* provide excellent illustrations for our purpose here. His data on the quantitative estimates of red and brown pigments are summarized in table 1. In order to compare the amount of variability within in-

TABLE 1

Data on the relative amounts of red and brown pigments present in various genotypic classes (after Nolte, 1959a, b).

Genotype	Red pigment		Brown pigment	
	Mean	C.V.	Mean	C.V.
w^+w^+	.8993	.0185	.0866	.0259
$w^{co}w^{co}$	.1454	.0484	.0182	.0756
$w^{e2}w^{e2}$	.0616	.0539	.0506	.0323
$w^{e3}w^{e3}$	.0542	.0511	.0091	.0879
$w^{sat}w^{sat}$	.0321	.0468	.1083	.0414
w^ww^w	.0308	.0561	.0342	.0412
$w^{bl}w^{bl}$	.0188	.1021	.0122	.0525
w^ew^e	.0142	.0451	.0182	.0598
$w^{ch}w^{ch}$	.0125	.0512	.0285	.0517
w^aw^a	.0108	.0533	.0089	.0719
$w^{a4}w^{a4}$	.0100	.0964	.0085	.0602
gg	.3380	.0548	.0485	.0858
g^2g^2	.1384	.1002	.0274	.0467
g^3g^3	.1900	.0556	.0409	.0939
g^4g^4	.2037	.0566	.0200	.0480

dividual phenotypic classes, the respective standard errors have been used to compute a coefficient of variation $\text{C.V.} = \left(\frac{\text{Standard Deviation}}{\text{Mean}}\right)$ in each case. It will be seen from these observations of Nolte that the hypomorphs have invariably a greater coefficient of variation than the wild type allele. Some of the findings of Wright (1949) on the melanin content of the hair of guinea pigs are also in agreement with the above model. Likewise, the observed variations in the expression of recessive genes for male sterility, or asynpasis, as against the more stable expression of their normal alleles indicate a larger variance for the recessive phenotype. It is likely, in fact, that the substrate-limit factor operates in general for a wide variety of characters although it may not be possible to analyze the phenotypic variability in all instances. For example, in the case of simple leaf condition in Cajanus, or rachilla hairiness in Hordeum, both determined by recessive factors, no obvious quantitative measure of the phenotype is available. It may also be pointed out that the greater variance for all recessive alleles need not follow the explanation proposed above, for neomorphs such as pheno-

deviant mutants apparently involve other developmental factors to account for their greater variation. Investigations have been undertaken on various ramifications of the model presented above.

We are indebted to Drs. B. P. Pal and A. B. Joshi for their helpful comments.

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ERRATA

In the first issue of 1961 (Vol. XCV, No. 880) the title page of the previous year was inadvertently used, resulting in an obsolete listing of Editors. The correct membership of the 1961 Editorial Board is as follows:

James T. Bonner, Princeton University
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Alfred E. Mirsky, Rockefeller Institute
Jack Schultz, Institute for Cancer Research
G. L. Stebbins, Jr., University of California
C. B. van Niel, Stanford University

L. B. Slobodkin requests a change in his paper on "Preliminary ideas for a predictive theory of ecology" (XCV, pp. 147–153). The line on figure 1 labeled ew and erroneously referred to in the text as the caloric value for egg white, is actually the accepted value for carbohydrate. This does not require alteration of any of the other statements in the paper.

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ADVICE TO AUTHORS

THE AMERICAN NATURALIST will welcome articles which contribute to the purposes outlined on the inside front cover.

Material intended for publication should be prepared to conform to the style in the current issues. It should be typewritten with double spacing, leaving a two inch margin at the right for editorial directions. Each table should be typed on a separate sheet. Footnotes to text statements should be avoided since they can usually be included in the text, parenthetically if necessary. Where unavoidable, they should be numbered consecutively and typed on a separate sheet, since they will be set in a different type size. Footnotes to tables are often necessary; they should be designated by asterisks, daggers and similar signs to avoid confusion with the numerals in the tables. Legends for figures should be typewritten on separate sheets.

Each article, except letters to the Editors, should contain a brief summary.

The "Literature Cited" assumes special importance in articles of the sort which THE AMERICAN NATURALIST hopes to publish. Authors are asked to give for each reference, the author or authors, the year of publication, full title and full citation, without abbreviation, of the journal, the volume number, the beginning and ending pages; or in the case of books, the edition number, the number of pages, and the name and address of the publisher. Current issues can be taken as samples of the style desired. Bibliographies which do not conform to the requirements above will be returned to the authors for correction. It is understood that general addresses will often not be accompanied by bibliographies.

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